Ecological risk assessment of pesticides in maize and tomato crops

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"A mesma esquizofrénica humanidade capaz de enviar instrumentos a um planeta para estudar a composição das suas rochas, assiste indiferente à morte de milhões de pessoas pela fome. Chega-se mais facilmente a Marte do que ao nosso próprio semelhante."

José Saramago, 1998

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Resumo Alargado

O aumento da população mundial tem vindo a colocar importantes desafios à produção agrícola mundial. Como praticar uma agricultura mais intensiva e ao mesmo tempo sustentável? Com a finalidade de dar resposta a este aparente paradoxo os países europeus tem empreendido grandes esforços para conhecerem melhor os seus ecossistemas e o impacto que a atividade agrícola sobre eles exerce, para assim os poder preservar. Por forma a rentabilizar ao máximo o rendimento das culturas, que sofrem perdas devido a infestantes, pragas e doenças, uma grande quantidade de produtos fitofarmacêuticos tem vindo a ser desenvolvida e utilizada ao longo do tempo. Porém apesar dos benefícios à produção, é hoje uma evidência que alguns destes produtos contém substâncias causadoras de impactos negativos nos ecossistemas aquáticos. O uso de pesticidas nos campos agrícolas não os confina ao seu local de aplicação, a entrada destes nos cursos de água pode ocorrer através da pulverização, drenagem, escorrimento ou fuga acidental. Uma vez presentes na água, os pesticidas provocam efeitos adversos (não totalmente conhecidos) nas populações de organismos que nela habitam, designados por organismos não-alvo. Tal ocorre devido à proximidade taxonómica destes organismos com algumas pragas e infestantes (organismos alvo). No entanto, efeitos temporários na estrutura e dimensão das populações decorrentes da exposição a pesticidas, podem ser consideradas aceitáveis se e só se, os impactos forem locais, temporários e a recuperação ecológica não for comprometida. Os organismos não-alvo como, parte integrante e fundamental do ecossistema agrícola, permitem a sua recuperação após contaminação através da capacidade que alguns apresentam para remoção das substâncias contaminantes. De forma a garantir o uso sustentável de pesticidas, é premente monitorizar, avaliar e reduzir os seus impactos negativos. Com o intuito de acautelar e melhorar o estado dos corpos de água europeus, tanto a nível ecológico como químico, a União Europeia desenvolveu a Diretiva Quadro da Água que entre outros processos, avalia o estado dos corpos de água através da monitorização de elementos indicadores da sua qualidade biológica como é o caso do zooplâncton. A avaliação de risco ecológico de pesticidas é um processo que permite avaliar a probabilidade de ocorrência de efeitos ecológicos adversos ou de que estes já estejam a acontecer, devido à exposição a um ou mais “stressors”. No entanto, devido à complexidade dos ecossistemas naturais, a capacidade de estimativa do processo de avaliação de risco (ARE) para múltiplas varáveis é severamente limitada. Embora estudos mais completos do processo de avaliação de risco de pesticidas tenham vindo a ser desenvolvidos, o fato destes serem maioritariamente realizados na Europa Central, tem afetado a extrapolação de resultados para outros países em que as condições climáticas são diferentes, como é o caso

de Portugal. A diferença de geografia implica diferenças no solo, condições climáticas e biota. Como tal, existe uma necessidade crescente de desenvolver o processo de ARE de pesticidas noutras regiões. Com o intuito de aumentar a relevância ecológica na avaliação de risco ambiental de pesticidas em condições Mediterrânicas, o presente estudo pretendeu estabelecer possíveis ligações entre a exposição a pesticidas e efeitos em organismos aquáticos não-alvo (zooplâncton) presentes em águas de rega de culturas de tomate e milho em condições de campo, assim como proceder à sua identificação uma vez que os conhecimentos sobre a taxonomia e ecologia locais são essenciais para que se possam produzir contribuições para avaliações de risco ambiental significativas. Esta dissertação encontra-se dividida em 4 capítulos organizados da seguinte forma:

- **Capítulo I** – Introdução: Abordagem sobre o tema da segurança alimentar e proteção ambiental no século XXI, apontando os desafios para uma agricultura ecológica e sustentável. Contextualização do uso de pesticidas no âmbito da gestão dos corpos de água, alertando para a necessidade e importância da proteção e estudo das comunidades de zooplâncton. Enquadramento da dissertação apresentado os seus objetivos como contributo para o melhoramento da avaliação de risco ambiental de pesticidas em Portugal.
- **Capítulo II** – Material e métodos – Caracterização da zona de estudo, apresentando duas das suas mais importantes culturas agrícolas (tomate e milho) e o seu enquadramento no estudo. Descrição detalhada das metodologias de colheita e identificação do material objeto de estudo e à seleção e caracterização dos pesticidas estudados.
- **Capítulo III** – Artigo: Apresentação e discussão dos resultados e conclusão sob formato de artigo. Este capítulo inclui uma descrição da amostragem, composição, abundância e riqueza em zooplâncton das amostras dos locais estudados. Assim como os possíveis efeitos dos pesticidas selecionados, sobre a ecologia do zooplâncton.
- **Capítulo IV** – Observações finais: Neste capítulo final são apresentadas algumas sugestões para estudos futuros e é seguido pelas referências bibliográficas consultadas.

Resumo

Foi realizado um estudo das comunidades de zooplâncton presentes na água de irrigação das culturas de milho e tomate em condições mediterrâneas portuguesas, a fim de vincular possíveis relações entre a exposição a pesticidas e as respostas biológicas. Este trabalho é uma contribuição para melhorar a relevância ecológica da Avaliação do Risco Ambiental de Pesticidas. Um total de 37 espécies de rotiferos e 2 famílias de cladóceros foram identificadas. Os principais componentes do zooplâncton em todos os locais de amostragem foram nauplios e rotíferos que parecem ser menos afetados pelos pesticidas. A concentração de 12 ug / l de clorpirifos reduz o número de macrozooplâncton, permitindo o aumento das densidades de rotiferos. Valores de 3,5-4,7 ug / l de clorantrinaprole e 0,96 ug / l de metribuzina parecem afetar negativamente o tamanho da comunidade de copépodos. As comunidades de Cladóceros e Ostracodes parecem diminuir quando os valores do glifosato estão na faixa de 2,3-3,9 ug / l. Os valores de glifosato (0,66 ug / l), Ampa (0,88 ug / l) e **Fosfato** (2,38 mg / l) parecem estar ligados a valores mais baixos de índice de riqueza de espécies.

Palavras-chave: Pesticidas, Zooplâncton, Culturas de Milho e Tomate, ERA, Ecologia.

Abstract

A study of zooplankton communities present in the irrigation water of maize and tomato crops under Portuguese Mediterranean conditions was carried out, to link possible relations between pesticide exposure and biological responses. This work is a contribution to improve the ecological relevance of Environmetal Risk Assessment of Pesticides. A total of 37 rotifer species and 2 cladoceran families were identified. The main zooplankton components in all sampling sites were nauplii and rotifers that seem to be less afffected by the pesticides. The concentration 12 ug/l of chlorpyrifos may reduce the number of macrozooplankton, allowing the raise of rotifer densities. Values of 3.5-4.7 ug/l of chlorantrinaprole and 0.96 ug/l of metribuzin seem to negatively affect the size of the copepod community. Cladoceran and Ostracod comunnities seem to decrease when glyphosate values are in the range of 2,3-3,9 ug/l. Values of glyphosate (0,66 ug/l), Ampa (0,88 ug/l) and **Phosphate** (2,38 mg/l) seem to be linked to lower species richness indexes.

Keywords: Pesticides, Zooplankton, Tomato and Maize crops, ERA, Ecology.

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Acronyms List

ABLGVFX – Associação de Beneficiários da Lezíria Grande de Vila Franca de Xira

BCPC - British Crop Production Council

BMP – Best Management Practices

CPL - Classification, Labelling and Packaging

DGAV – Direção Geral de Alimentação e Veterinária

E.U – European Union

EC – European Commission

EIA –Ecological Intensive Agriculture

ERA - Ecological Risk Assessment

FAO – Food and Agriculture Organization

INE – Instituto Nacional de Estatística

POM – Particulate organic matter

UN – United Nations

WFD – Water Framework directive

CHAPTER I

General Introduction

1. Food safety and environmental protection in the XXI century. The challenge of Sustainable/Ecological Intensive Agriculture

The United Nations goal to eradicate extreme poverty and hunger all over the world is unfortunately still far behind from being a reality. This organization established as a target to halve, between 1990 and 2015, the proportion of people who suffer from hunger. The fact is that even though the proportion of undernourished people globally decreased from 23.2% in 1990-1992 to 14.9% in 2010-2012, 870 million people are still going hungry. (FAO, 2015).

According to FAO by 2050, the world's population will reach 9.1 billion. The developing countries will be the ones heading this growth. The urban population will continue to grow reaching about 70%, with much higher incomes than it has today. China, Brazil and India, for example, in the last years have raised their middle-class population at notable rates and with this arise in numbers and income, came the demand for more and diverse food products that need to be produced in the global market, since many countries still depend on it to assure their food security. FAO claims that food production (net of food used for biofuels) must increase 70% compared to 2009 numbers. Annual cereal production will need to rise about 3 billion tons from 2.1 billion in 2009 and annual meat production will need to rise by over 200 million tons to reach 470 million (FAO, 2015).

The development model that has been used in the past, has greatly improved production volumes per both area and labor unit. However, this growth has until recently been assessed by only land, capital and intermediate inputs factors. This assessment has ignored for years the quantity of natural resources used for agricultural production. This is mainly due to ignorance of the limits of natural resources exploitation when the economic model was developed in the nineteenth and early twentieth century (Fourastié, 1978)

During decades, natural resources needed for agriculture have been given a second role not considering that they are limited and passive of degradation. Ironically, intensive agriculture has been a "victim" of its own production factors due to their disturbance effect on the ecological balance. By disrupting the ecosystems natural balance, the pesticides contribute to the raise of pest populations and diseases leading to an increase of the frequency and amounts of usage (Daam *et al.,* 2011).

Therefore, the watercourses that surround the agricultural fields have been contaminated due to pesticide spraying, drainage, runoff and accidental leaking (Daam *et al.,* 2011).

The ability of soil and water to recover from pesticide contamination is mainly dependent on the existence of an abundant and diverse microbial community with the ability to remove contaminants (Barra Caracciolo *et al*. 2013).

The association of climate changes and intensive and unsustainable agricultural practices is contributing to soil degradation and loss of biodiversity (Jeffery *et al*. 2010; Turbé et al. 2010).

The increase of drought and flooding frequency and amplitude, temperature increase, loss of natural water depuration, soil erosion leading to loss of carbon content, increase of pest events, changes in the plants phenology, increase sensitivity of crops to stress and diseases, among others (Fisher *et al.* 2005; Howden *et al*. 2007), are the manifestation and consequence of the global climate change that is affecting many ecosystems and societies throughout the world. The impact of climate changes will affect all levels of biological organization, from individual species to entire ecosystems (Fisher *et al.* 2005; Howden *et al*. 2007), . A considerable amount of studies regarding this subject showed the following results:

- Enhanced toxicity for organisms not adapted to increased temperatures (Ferrando *et al.* 1987; Lydy *et al.* 1999; Prato *et al*. 2008);

- Subtle changes in environmental conditions or key species (e.g. (Levinsky *et al*. 2007)) as well as impacts on ecological networks (Meerhoff *et al*. 2007; Woodward et al. 2010; Ledger *et al*. 2013);

- Exposure of freshwater ecosystems to eutrophication processes due to high temperatures that will likely increase the overall metabolism and nutrient uptake of these freshwater ecosystems (Demars *et al.* 2011);

- Collapse of the entire food web structure in the absence of apex predators under drought conditions (Ledger *et al.* 2013).

- Increased risk of pesticides exposure to the aquatic biota due to the Increase of surface run-off events in periods of high agricultural production (Babut *et al*, 2013);

- Rise in the biodegradation rates of chemicals for both aquatic and terrestrial ecosystems and in freshwater ecosystems, due to the increase of temperature reducing their ability to cope with increased temperature levels (Friberg *et al*. 2009; Jeppesen et al. 2010).

In opposition, there is enough convincing evidence that intensive sustainable farming systems can bring economic benefits to farmers and enterprises as well as environmental conservation (Pretty, 1997). This is possible by the lowering of external farming systems inputs, regeneration

of local resources, the addition of value to the agricultural production and maintaining local communities' surpluses (Pretty, 1997). Although there are already, many success cases over the world to achieve a more sustainable use of the land these cases must be replicated. For this to be possible educational communities (communities of participatory learners) are required, not simply distributors of ready-made technologies (Pretty, 1997). So, a new challenge for today's farmers, enterprises and agronomists: new concepts, values, methods, behavior – in other words, a new professionalism (Pretty, 1997).

According to Pretty (1995) the goals for a Sustainable Agriculture should be the following:

- A thorough integration of natural processes such as nutrient cycling, nitrogen fixation, and pest-predator relationships into agricultural production processes, so ensuring profitable and efficient food production;

- A minimization of the use of those external and non-renewable inputs with the potential to damage the environment or harm the health of farmers and consumers, and a targeted use of the remaining inputs used with a view to minimize costs;

- The full participation of farmers and people living in the rural areas in all processes of problem analysis, and technology development, adaptation and extension;

- A more equitable access to productive resources and opportunities, and progress towards more socially just forms of agriculture;

- A greater productive use of local knowledge and practices, including innovative approaches not yet fully understood by scientists or widely adopted by farmers;

- The enhancement of wildlife and other public goods of the countryside;

- An increase in self-reliance amongst farmers and people who inhabit rural areas;

- An improvement in the march between cropping patterns and the productive potential and environmental constraints of climate and landscape to ensure long-term sustainability, of current production levels.

After the Grenelle Environment forum (2007), Michel Griffon initiated the concept of Ecological Intensive Agriculture (EIA). This concept "per se" seems to be paradoxical but the author ensures that an ecologically friendly, sustainable, more productive and fair agricultural development that ensures the worldwide demand for food, while improving environment quality is possible. It is

assumed that a more complex management of the agricultural techniques as well as landscaping needs to be more complex than in conventional farming. However, this term still pursues conventional farming goals: maximizing yields and farmers' incomes. For this to happen animals and plants with important intrinsically potential of production (thus excluding transgenic organism's), as well as production conditions that maximize the expression of this potential are fundamental, including adding the necessary quantities of inputs for its growth and development (Griffon (2007, 2006)). Therefore, the future challenge is reclaiming the optimization features of ecosystems which mean the reduction of usage of synthetic inputs and non-renewable resources without compromising the viability of farms and their production levels. Natural resources must then be placed in the center of the economic functioning of the agricultural sector considering them factors of production and it is this assumption that distinguishes the approach of EIA from other concepts of agriculture (El Ghali *et al*. 2012).

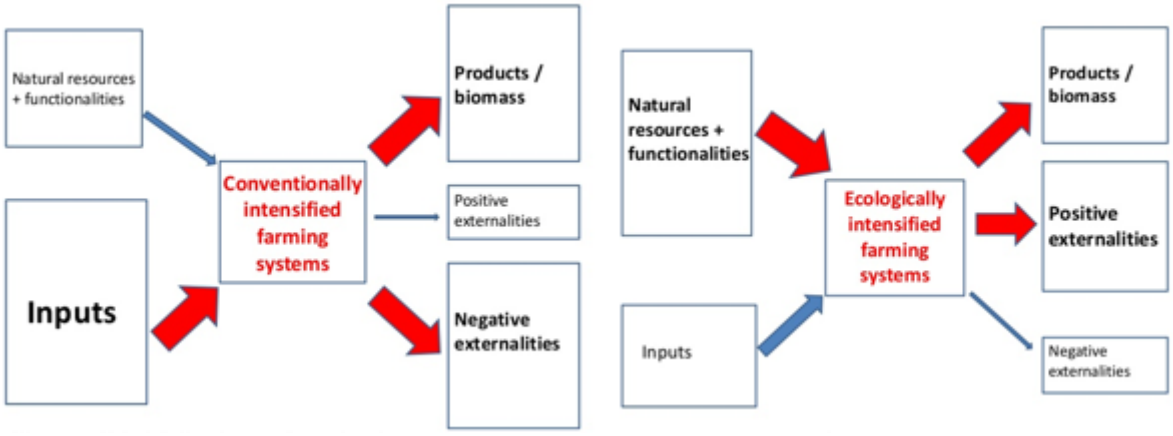


Figure I.1 – Compared intensity of cropping systems (Adapted from M. Griffon 2013)

Exposing this concept is very pertinent due to two main reasons. First, this concept does not put preservation of the environment and the maintenance or improve of agricultural yields in opposite sides. Second because its main goal isn't to introduce a mandatory list of changes but instead a process of gradual evolution of practices implemented by farmers engaged in this innovation process.

According to Griffon, (2007) EIA approach is based on two pillars:

- New technology: Production techniques are strongly inspired by the knowledge of the mechanisms of life and the functioning of the nature. An ecologically extensive technology bases on four elements to consider simultaneously i) the quantitative effort where every region of the world must increase its cultivated surfaces or its yields without affecting irreversibly the biodiversity by massive clearings. Europe for example has to maintain its returns, reduce its consumption of fertilizer, pesticides and energy by reducing environmental damage ii) the qualitative effort corresponds to the improvement of the sanitary and gustative quality of food. The sense of change is the phasing out of the standard qualities for the benefit of diversification of products, iii) the production of ecological services namely: maintain the natural cycles such as water, carbon by sequestration of the organic matter in soils and iv) adaptation to climate change.

- A new agricultural policy assuming a redistribution of natural capital, significant investments in ecological restoration, more stable prices, markets and provided tariff protection, when the market competition can only lead to a scenario of under-nutrition and stagnation of food economics.

In sum, the EIA approach leaves the achievement of its environmental and economic goals in a very complex position. Their success will depend on three decisive points: the first one concerns the characterization of the natural resources and the identification of their relationship with agricultural yields. Meaning that the "input-output" relation must be identified in order to determine the production function (El Ghali *et al.* 2012). The second one regards the contribution of the natural resources regarding the overall efficiency and the environmental and economic efficiency of the production systems (El Ghali *et al.* 2012). The last one regards the research of new practices and technologies that are able to enhance the functioning of natural resources and improve agricultural yields and economic performance (El Ghali *et al.* 2012).

2. Pesticide use and water resources. Protection of non-target species.

2.1 Brief analysis of pesticide sales in Portugal

The pesticide definition from The Pesticide Manual of the British Crop Production Council, refers to as “the substance or substance mix used to prevent, destroy or attenuate pests or when used as plant growth regulator, defoliant or desiccant” (BCPC,2015).

The sale knowledge of plant protection products contributes for the evaluation of the introduction of these products on the national agricultural territory as well their potential environmental impact. The use of these products varies according to weather conditions, phytosanitary problems and their market price (INE, 2015)

The sales structure of Plant Protection Products in Portugal from 2011 to 2013 is shown on Table I.1

Table I.1 - Plant Protection Products Sales (INE, 2015)

	Unit	2011	2012	2013
Plant protection products sales by type of fuction				
Fungicides	t a.s	9,975	8,517	7,203
- **Sulphur**	t a.s	6,697	6,081	4,905
Herbicides	t a.s	1,996	1,769	1,611
Insecticides and acaricides	t a.s	880	811	747
Others (a)	t a.s	1,175	1,365	566
Total sales	t a.s	14,026	12,462	10,127
	Kg a.s/ha	3,8	3,4	2,7
	Kg a.s/ha	2,0	1,7	1,4

Note: t a.s: tons of active substance; a) Includes soil fumigants, growth regulators, rodenticides and other

Through the analysis of the sales structure it is possible to highlight the fungicide group as the more important, representing in the year 2013 approximately 71,1% of the whole sales volume, followed by herbicides (15,9%) and insecticides and acaricides (7,4%) (INE, 2015).It is important to mention that sulphur, was responsible for 68,1% of the fungicides total sales volume (71,4% in 2012) and for 48,4% of the total volume of plant protection products (48,8% in 2012) (INE, 2015) . The decrease that was verified in the commercialized quantities of this substance in 2013 (less 19,3%), in comparison with 2012, was the main promotor of the decrease in the plant production products in that period (INE, 2015). The amount of Plant protection products used per ha in the year 2011 was 3,8 Kg a.s and decreased to 2,7 Kg a.s in 2013. Considering all Plant

protection products apart from Sulphur the amount used per ha in 2011 was 2 Kg a.s and it decreased to 1,4 Kg s.a in 2013 (INE, 2015).

2.2. Legislative requirements regarding Sustainable Pesticide use and Water Management

2.2.1 Sustainable Use and Market Launch of Pesticides

The sustainable use of Pesticides in Agriculture is defined by the European Commission as the use of pesticides without irreversible effects in the natural systems and that doesn't cause acute or chronical effects to Men, animals and environment (Amaro, 2003; EC, 2001). A sustainable use means reduction of pesticides to the maximum, use restriction or replacement of the most harmful and adoption of the precautionary principle in the homologation processes (Amaro, 2003; EC, 2001).

The introduction of Plant Protection Products in the market is preceded by a technical and scientific evaluation of the risk for Man as an applicator and consumer of agricultural products that have been treated, for animals, for the environment and non-target species (DGAV, 2015). Permits for market commercialization are only conceded to products that regarding these guidelines and that when utilized according to instructions do not have harmful effects on human and animal health (DGAV, 2015). Also, that shouldn't be able to exercise any kind of harmful influence in the environment and that of course show proper demonstration of effectiveness regarding their proposed usage (DGAV, 2015). The successive scientific knowledge that is being produced worldwide is framed in the evaluation systems, through rising demands with the goal to bring the best benefits for Agriculture without harming the environment and causing public health issues (DGAV, 2015).

Recently was published innovative communitarian legislation regarding plant protection products, constituting what is commonly known in Portuguese as "pacote pesticidas" (DGAV, 2013). Thus, together with the Directive n. º 2009/128/EC, was published the Regulation (EC) n. º 1107/2009 of 21st of October (that substitutes Directives 79/117/CEE and 91/414/CEE), regarding the market launch of Plant Protection Products, establishing rules applicable to:

- Authorization of the commercial forms of Plant Protection Products, their market launch, use and control in the community;
- Approval of active substances, fitotoxicity protectors and synergistic agents that are contained or constitute the Plant Protection Products;
- Adjuvants and coformulates.

The regulation intends to enforce the exigency level regarding protection of human, animal and environment health (EC, 2009; DGAV, 2012). Also, to improve internal market dynamics trough harmonization of norms regarding the market launch of Plant Protection Products, improving simultaneous Agricultural production (EC, 2009; DGAV, 2012). This regulation also intended to eliminate as far as possible barriers to the commerce of Plant Protection Products that arise trough the existence of different Member States realities regarding Plant Protection, thus establishing harmonized rules for active substance approval and Plant Protection Products launch in the market and rules for the mutual recognition of authorizations and parallel commerce with the goal to raise free circulation of this products and guarantee their availability in all Member States (EC, 2009; DGAV, 2012).

The Directive 2009/128/EC of the European Parliament and Council 21st of October, regarding the use of Plant Protection Products, establishes a community level frame of action for the sustainable use of pesticides, trough reduction of risks and effects on human health and in the environment deriving from their use, promoting Integrated Pest Management and alternative approaches or techniques (EC, 2009; DGAV, 2013). . The Directive can't prevent Member States from applying the so called precautionary principle, to restrain or prohibit the use of pesticides in certain areas or under specific circumstances (EC, 2009; DGAV, 2013).

It was recently published the national law nº 26/2013, 11th April into force on 26 of November 2015, regulating the activities of distribution, sales and use of Plant Protection Products of Professional use and sets the monitoring procedures for their use. This law among the Decree-law n. º 86/2010 of 15th July, transpose the Directive 2009/128/Ec for internal legal order forming the new legal framework concerning sale and use of Plant Protection Products (European Directive 26/2013; Decree-law 86/2010; DGAV, 2013).

In order to achieve a sustainable use of Pesticides, new application technologies are being developed, and they consider many practices, particularly in a landscape context: respect for label recommendations, appropriate application planning, respect for label recommendations, the use of weather and pest forecasting services, an extra water tank for in-field equipment cleaning, using spray drift reduction technology, e.g. anti-drift nozzles, remnant management with bio-purification systems, implementing and respecting multifunctional field margins (Syngenta, 2012). These practices must be accompanied by a proactive on-farm water management to secure the water resources and be able to meet the European requirements for the sustainable use of pesticides. In the last years there have been quick technology development in technology and that makes it mandatory for farmers and agronomists to be

continually updated and trained on the handling, use and maintenance of pesticide application equipment's (Syngenta, 2012).

The challenge of reducing the risks and impacts of pesticide use in Europe remains in the use approach that shouldn't be too restrictive and focused only on the reduction of used doses because food safety must be guaranteed (Syngenta, 2012).

2.2.2 Water Management

Contamination of surface and ground water may occur by spray drift, drain flow, run off and field leaching. In the last years it has been a key goal of E.U legislation to further improve the way we protect surface and ground water, including those that are sources of drinking water. For this purpose, the European Parliament and the European Union council, created in 2000 a new legal framework that establishes a framework for community action regarding water politics, the Water Framework Directive **(**Directive 2000/60/EC). This Directive considers the water resource as a heritage that must be protected thus establishing a big change in the way the E.U sees the evaluation, control and management of all ground and surface water based on their chemical and ecological status (EC, 2000; CCE, 2002). The principles and goals mentioned in the Directive 91/414/CEE regarding pesticides were translated into goals for all water sources (CCE, 2002).

The Water Framework Directive (WFD) incorporated the Directives 75/440/CEE (regarding surface water), 76/464/CEE (regarding discharges of hazard substances) and 80/68/CEE (regarding ground water), all these mentioned Directives were revoked in 2013 since WFD was in force (CCE, 2002).

With the purpose of maintaining and improving water environment, WFD sets the framework for the management of superficial and ground water to:

- Prevent the degradation of water resources and improving and protecting the status of aquatic, land and wet zones ecosystems;
- Promote a sustainable use of water based on a long-term protection of the available hydric resources;
- Obtain an enhanced protection and improvement of the aquatic environment through gradual reduction measures and the cessation/elimination of discharges, emissions and loss of priority substances;
- Assure the gradual reduction of groundwater pollution and avoid the aggravation of its pollution.

In order to combat water pollution, the substances with priority character that constituted a significant risk for the aquatic environment were identified. According to 2013/39/E.U Directive, there are 45 priority substances identified, this Directive also establishes environmental quality standards (EQCSs) for these substances in surface waters and confirmed their designation as priority or priority hazardous substances. According to Annex V, point 1.4.3 of the WFD and Article 1 of the EQSD, good chemical status is reached for a water body when it complies with the EQSs for all the priority substances and other pollutants listed in Annex I of the EQSD (EC, 2014).

According to the new "Plano Nacional da Água", only 52% of the Portuguese water courses are in a "good state" according to E. U's legislation. The report explains that less than half the country's rivers and bayou's is still polluted or reasonably changed. The Water Framework Directive previews that a good state of rivers, lakes and bayous would be reached by 2015. But admitted an extension of the deadline until 2027, unjustified cases, if it was technically impossible to achieve the goals in 15 years or if the costs where to high.

The adoption of Best management practices such as field margins, optimized irrigation systems and best in class application technology us crucial to help prevent this problem as well as improving agricultural productivity. This contamination can be prevented by many ways, for example with proper management of spray equipment, regarding filling and cleaning processes. (Syngenta 2012).

2.3 Non-target species the importance of zooplankton and it's recovery

When Plant protection products are used for crop protection, they are directly introduced in the environment having the potential to contaminate soil and water causing mortality in both land and aquatic non-target species (DGAV, 2015). After the application they are subject to distribution processes, biological degradation and dissipation by means of different climate agents that allow the reduction of the residues levels on the different environmental compartments (DGAV, 2015).. On the other hand, being products with an inherent certain level of toxicity the risk they represent to the environment depends on the conjugation the residue level that is present in a certain environmental compartment and its inherent toxicity. (DGAV, 2015).

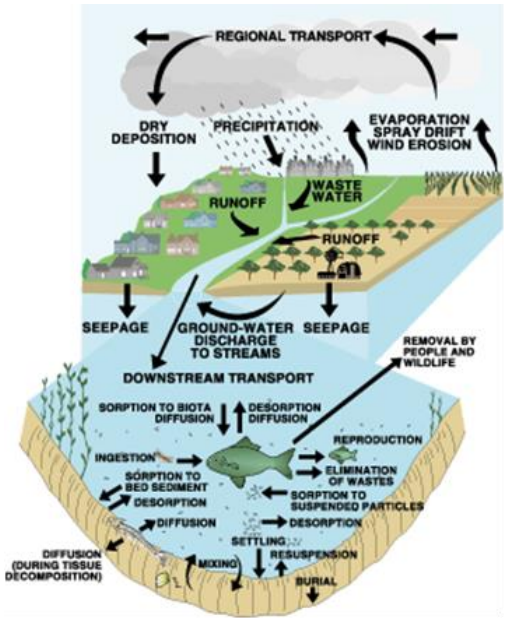


Figure I.2 - Pesticide movement in the hydrologic cycle including pesticide movement to and from sediment and aquatic biota within the stream. Modified from Majewski and Capel (1995).

The biological activities of pesticides do not remain restricted to a target organism but are also extended to non-target organisms that often play important roles in the ecosystem they inhabit (Netrawali et al., 1986).

According to (Hanazato, 2001) the effects of pesticides on freshwater zooplankton are multiple: toxicity is shown to vary depending on animal species, genotype, life stage, and size at birth. Natural stresses such as food shortage, oxygen depletion and odors of potential predators can

also affect toxicity (Hanazato, 2001). Populations in the growth phase are vulnerable to pesticides but have the potential to recover rapidly from the damage (Hanazato, 2001). Pesticides may affect the population dynamics by controlling individual survival and reproduction, and by altering the sex ratio. Furthermore, toxic chemicals may control predation risk by changing swimming behavior and body morphology, and this in turn influences the population dynamics (Hanazato, 2001) . Many zooplankton organisms display morphological and behavioral responses to predators when exposed to their odor-producing chemicals. However, pesticides induce a maladaptive response to predator odor, and this poses an ecological risk (Hanazato, 2001). The following patterns are recognized as effects of pesticides at the community and ecosystem levels: (1) induction of dominance by small species; (2) a decrease of species richness and diversity; and (3) elongation of the food chain and reduction of energy transfer efficiency from primary producers to top predators (Hanazato, 2001) .

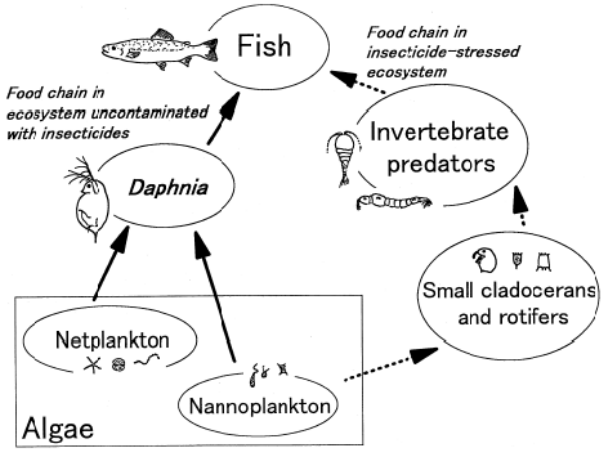


Figure I.3: The main pathways of carbon and energy flow from algae through zooplankton to fish in lake ecosystems contaminated and uncontaminated with pesticides. Redrawn from Hanazato, 1998.

Aquatic ecosystems are well known for their organism diversity and abundance. Lakes, rivers and reservoirs, regardless of their size they are inhabited by many planktonic organisms (Margalef, 1984). These organisms are the base of the food chain and due to their high metabolism capacity, they can influence fundamental ecological processes such as nutrient cycling and the magnitude of the biological production (Edmondson, 1959). Thus, their knowledge and preservation are fundamental for a balanced and sustainable agricultural ecosystem. Among these organisms is Zooplankton (from the Greek zoo = animal, planktos = floating), Zooplankton is constituted by primary consumers (herbivores) and predators of different trophic levels (Wetzel, 1993).

According to its size zooplankton is divided in (Wetzel, 1993):

- Picoplankton (0.2-2 µm, mostly bacteria);
- Nanoplankton (2-20 µm, mostly protozoa);
- Microplankton (20-200 µm, protozoa, rotifers and juveniles of microcrustacea);
- Macroplankton (>200 µm, larger protozoa, some rotifers and most microcrustacea).

The habitat categories are (Wetzel, 1993):

- Limnoplankton (the plankton of fresh waters especially of lakes);
- Heleoplankton (plankton typical of small bodies of still fresh water);
- Potamoplankton (plankton living in freshwater streams).

According to (Wetzel, 1993) the three dominant groups of zooplankton in freshwater ecosystems are: Cladocera, Copepods and Rotifers. Ostracods are also very representative (Horne et al., 2002).

Cladocera are small branchiopod crustaceans also known as "water fleas". They are a significant component of the microfaunal food webs, e.g. as grazers of phytoplankton, as part of the diet of macroinvertebrates or juvenile fish. In size they range from < 250 µm in the chydorid genus Alonella to 4-6 mm in the dahpniid genera Daphnia and Simocephalus (Shiel, 1995). To evaluate pesticide toxicity, standardized acute and chronic toxicity tests have been intensively conducted using cladocerans, in particular the species Daphnia (OECD, 1981; ASTM, 1994).

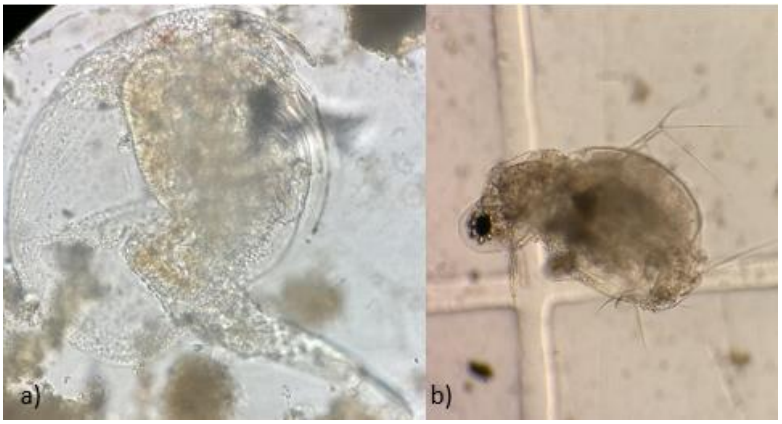


Figure I.4: Two Cladocera from different families [a) Family *Chydoridae* b) Family *Moinidae*] (Oliveira, 2015)

The **Copepoda** subclass is the largest class of the Crustacea, and it has a predominantly marine affinity, with a large group of species that are fish parasites. Three free-living orders with freshwater representatives are: Calanoida, Cyclopoida and Harpacticoida. Calanoida and Cyclopoida are both common in small ponds. Harpacticoids are benthic in habitat, and rarely collected in open water. Copepods are very important in the aquatic food webs specially their juvenile stages (naupli or copepodites) as a major food supply for young fish (Shiel, 1995).

Figure I.5: Different Copepod life stages [a) Nauplius; b) Copepodite; c) Adult female with eggs (Oliveira, 2015).

Forming a separate phylum **Rotifers**, small organisms (up to 2mm) have at least 2000 species (Howey 1999). They have a multicellular organization and at least the females possess a primitive brain (Hingley, 1993). Known also as wheel animals because of their locomotor systems, they are somehow resistant to extended periods of drought. Rotifers choose substrates according to several factors: water temperature, oxygen content, trophic levels, chemistry, food availability, and presence/absence of predators (Pejler & Bērziņš, 1989). Rotifers may disperse by means of their resting eggs and their biogeography has been subject of intense controversy (Dumont, 1980, 1983; De Ridder, 1981; Shiel et al., 1989; De Manuel et al. 1992). Rotifers have three types of individuals: mictic (mixing) females, amictic females (not reproducing sexually), and males.

Figure I.6: Different rotifer species a) *Platyias quadricornis* (Ehrenberg), b) *Keratella tropica* (Apstein), c) *Filinia brachiata* (Rousselet) (Oliveira, 2015).

As generalist feeders, rotifers feed on an array of food items in their natural habitats. There are reports on transfer of pollutants from sediment to rotifers and toxicity of sediment toxicants in rotifers (Cargouet *et al.,* 2004; Garcia *et al*., 2007). This suggests that rotifers can suitably be used to measure the transfer of pollutants from one environmental compartment and trophic level to another in aquatic systems. Rotifers generally show effects of bioaccumulation and biomagnification (Dahms *et al*., 2010). Although rotifers are small, they might add substantially to the secondary production in aquatic systems, because of their large population size (Wallace, 2002) that is coupled with high turnover rates due to fast growth and at times parthenogenetic reproduction (Snell and Janssen, 1995). They can regenerate nutrients from plankton and particulate organic matter (POM), efficiently used as food due to their high metabolic rates (Wallace et al., 2006). Since they are very easy to find and cultivate, rotifers became a useful model system for studies in aquatic ecology (Gomez, 2005) and ecotoxicology (Kaneko *et al.,* 2005). Other characteristics that make these organisms appropriate for aquatic ecotoxicity testing and as sensitive bioindicators of water quality changes overtime (Kotani *et al*., 2001) are: small size, sensitivity to a vast number of toxic substances, predominantly parthenogenetic reproduction (providing genetically and phenologically identical clones for testing), availability of culture techniques, high population density and rapid population growth rates (Hagiwara et al., 1997; Hagiwara *et al,* 2007). Ecotoxicological testing with rotifers generally includes evaluations under both laboratory and field conditions (Snell and Joaquim-Justo, 2007). Field testing is primarily based on variations in species diversity indices, body size and population densities (Marcial *et al.*, 2005), these evaluations should be supported by hydrological data collected simultaneously. Commonly the toxic substances that are tested with rotifers are natural toxins, pesticides, and heavy metals (Marcial *et al*., 2005). For all that's been said, Rotifers are expected to play an increasing role in ecotoxicology and environmental genomics (Denekamp *et al.,* 2009; Suga et al., 2007a,b, 2008) and may assume an equally or more important status than other invertebrate taxa, such as arthropod cladocerans (Dobsikova, 2004).

Ostracods are small crustaceans ranging in length from 0.08 to 3 mm, or more. Their entire body is encased in a bivalved, calcified carapace which can be smoothed to various ornaments. The two valves are joined by a dorsal hinge and by a closing muscle (Keyser, 1988). The body is unsegmented and has a reduced number of limbs. The head is larger than both the thorax and abdomen combined. The ostracods bear normally eight pairs of appendages: the first and second antennae, mandible, maxilla and three additional thoracopods, with the abdomen terminating in a pair of caudal rami (Keyser, 1988). The three thoracopods are often used as walking or cleaning legs. The genital organs are situated between the last thoracopods and the

caudal rami (Keyser, 1988). The large male copulatory organs which fill up the posterior half of the whole carapace are often obvious (Keyser, 1988). Some 65,000 living and fossil species have been described and grouped into several orders (Horne *et al.,* 2002).

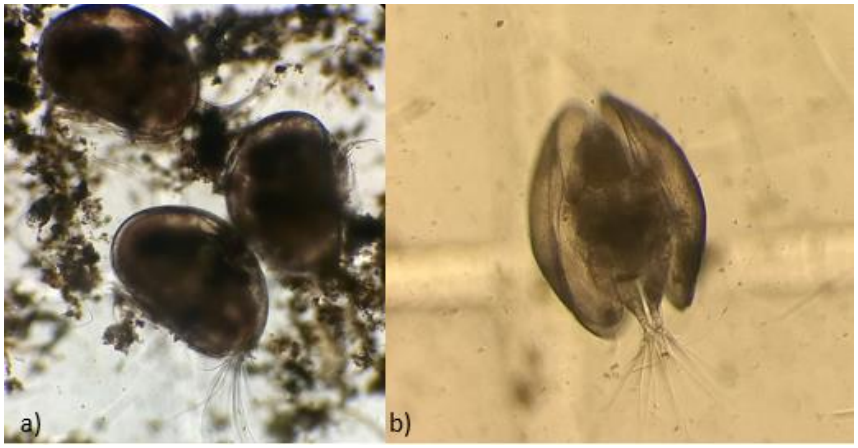


Figure I.7: Ostracods [a) Group of ostracods; b) Ostracod with open shell], (Oliveira, 2015).

3. Ecological Risk Assessment of Pesticides. Placing this thesis into context

The Ecological Risk Assessment is a process that evaluates the likelihood that adverse ecological effects may occur or are already occurring because of exposure to one or more stressors (U.S. EPA, 1992). This process is used to systematically evaluate and organize data, information, assumptions, and uncertainties to help the understanding and prediction of the relationships between stressors and ecological effects in a way that is useful for environmental decision making (U.S. EPA, 1992). The ecological risk assessment process is based on two major elements: characterization of effects and characterization of exposure. These provide the focus for conducting the three phases of risk assessment: problem formulation, analysis, and risk characterization. During the analysis phase, data are evaluated to determine how exposure to stressors is likely to occur (characterization of exposure) and, given this exposure, the potential and type of ecological effects that can be expected (characterization of ecological effects) (EPA. 1998 - Guidelines for Ecological Risk Assessment). To plan this kind of assessment there is a lot of considerations to be done. Sometimes the best theoretical model doesn't fit to nature constraints, lack of valid data and scientific understanding, expertise, time and financial resources. Estimating the effects of multiple stressors on natural populations of organisms is very challenging. ERA is dependent on ecotoxicological data obtained via laboratory toxicity studies which by their design, sacrifice ecological realism for the sake of reproducibility, commonly describing the effects of a limited number of toxicants on a limited number of species with strict control of environmental conditions (Cairns, 1983). Even though these experiments are needed, the ERA process needs to extrapolate ecosystem level effects from ecotoxicological studies conducted at low levels of biological organization. But with such extrapolations there are numerous assumptions and uncertainties associated. Model test organisms may not be indicative or representative of indigenous species (Reynoldson *et al*., 1994; LaPoint and Waller, 2000). In natural conditions, organisms may be exposed to complex mixtures instead of individual toxicants (Barnthouse *et al*., 2000). The natural variation of physical and chemical environmental conditions is largely overlooked as a source of uncertainty in risk estimates (Lozano and Pratt, 1994; Preston *et al*., 2001). Natural ecosystems are very complex, and this factor limits severely the capacity of ERA to estimate the associated risk of multiple stressors (Moore and Bartell, 2000). Recent attempts have been made in order to integrate multiple risk estimates (Lozano and Pratt, 1994; Preston *et al*., 2001), but these calculations commonly assume additivity among stressors despite synergistic or antagonistic interactions are frequent (Folt *et al*., 1999). In a similar way, despite the fact of the effects of water quality, such as pH or dissolved oxygen, have been known for many years (Sprague, 1995), there is frequently

insufficient data to account for all such modifying factors in an integrated way. Consequently, ERA's focus very often solely on the effects of individual toxicants (Solomon *et al*., 1996; Hall *et al.*, 1998; Cardwell *et al*., 1999), providing little understanding of net effects or proportional risk regarding other stressors.

Higher tier studies for the environmental risk assessment (ERA) of pesticides in Europe have been mainly performed in Central Europe and their results extrapolated to other countries whit different climates, including the Mediterranean (López-Mancisidor *et al*., 2008; Daam *et al*., 2011) where soil, climatic conditions and biota are substantially different, thus possibly leading to risk misestimates (Brock, *et al*., 2010; Daam *et al*., 2011; López-Mancisidor *et al*., 2008; Ramos *et al*., 2000; Vanderborght *et al*., 2010). As such, there is an increasing necessity to develop or improve scenarios for ERA of pesticides in this region. The European Food Safety Authority (EFSA) took these concerns into consideration during current revisions of the existing legislation and new uprising topics trough the incorporation of Mediterranean scenarios (EFSA, 2010; 2012). The EU is revising the ERA procedures for pesticides to further update the ecotoxicological risk assessment guidance documents SANCO/3268/2001 and SANCO/10329/2002 (EC, 2002) suggesting the definition of specific protection goals at a population level for specific groups of organisms (microbes, algae, non-target vascular plants, aquatic invertebrates, terrestrial non-target arthropods, non-arthropod invertebrates and vertebrates) that play a key role in the ecosystems and are potentially impacted by pesticide use in agricultural landscapes (EFSA, 2010). Temporary impacts on population size or structure resulting from pesticide exposure may be considered acceptable if the impacts are temporary, local, and recovery occurs (Nienstedt *et al*., 2012). Ecological Recovery is the extent of return of a population or community to relevant aspect(s) of its previous condition or to the status of a control treatment or a reference site, either from outside or from within the affected system (EPPO 2003; US EPA 2015). Full recovery is reached when there is any or only a negligible difference in the properties of the previously affected population or community and that of the control treatment, reference site or the status before the pesticide application for a longer period. Recovery can be internal (i.e., from within the affected area by reproduction), or external (usually termed recolonization; i.e., from outside the affected area by individuals immigrating and thereby increasing the affected or starting a new population) (Liess *et al.,* 2013). Ecological Recovery is essential in ERA of Pesticides to assess if the exposure of organisms to a pesticide or mixture of pesticides present in the water bodies along time is compromising or not the ecosystem.

To validate and refine the ERA of pesticides the collection of national field data despite the fact of the limitations posed by natural and spatial variation of zooplankton populations, background levels of contaminants and the expenses of working in larger scales, assumes great relevance. Field studies offer advantages over laboratory toxicity studies, first because the observed field effects are the result of both direct and indirect effects of stressors, so the ecosystem level effects are directly observed rather than extrapolated trough data manipulation. Second the number of stressors that can be considered is much wider than those for which ecotoxicological data exists.

3.1. Thesis main goals

This thesis is integrated in the Doctoral Program of a Researcher still in course that has as main objectives:

i) Increase our understanding of the risk evaluation of environmental realistic pesticide mixtures. The link between results obtained by microcosms with the situation in the field needs to be strengthened, and it is clearly needed for the validation of SSD predictions regarding the effects of toxicant mixtures on biological communities in the field and the relation of effects with the environmental context.

ii) Address the lack of model ecosystem studies in South Europe and consequently increase our knowledge on pesticide fate, their direct and indirect effects on ecosystem structure and functioning as well as the recovery potential of impaired ecosystems

iii) Aim the creation of a scientific basis for criteria, linkage pesticide exposure and effects under relevant South European conditions achieving ecological water protection."

Working with field data is very demanding since it's difficult to clearly link exposure to possible effects given the number of variables that can influence zooplankton ecology so, a carefully developed research plan was conceived with two main goals:

1- To identify to the maximum extent possible, all zooplankton taxa present in the studied area, thus providing information about the organisms present which can be helpful for other works in terms of identification and data comparison.

2- To establish possible links between the effects of zooplankton exposure to pesticides during two different crop cycles under Mediterranean conditions using biological indexes and multivariate statistical analysis so, some contributions for the improvement of ERA of Pesticides for the aquatic ecosystems under such conditions can be given.

CHAPTER II

General Methodology

1. Description of the study site

Lezíria Grande de Vila Franca de Xira is one of the most important national agricultural parcel located 25 quilometers north of Lisbon, surrounded by the rivers Tejo and Sorraia who border it from the East and West in the countys of Vila Franca de Xira and Azambuja. The total agricultural area of 13 420 ha is divided in half by the nacional road number 10, which connects Vila Franca de Xira to Porto. The two resulting areas are called “Lezíria Norte” with 6 620 ha of cultivated area and “Lezíria Sul” with 6 800 ha (DGADR, 2014).

“Lezíria Sul” is part of a national natural reserve “Reserva Natural do Estuário do Tejo” which is considered to be the largest and most important wet zone of Portugal and one of the most important in Europe due to its richness in migratory waterfowls. The cultivated areas are an essential winter refugee and nesting place for these birds (EVOA, 2014).

The first hydric intervention in the area was in 1910, when “Canal Principal”, was constructed crossing lengthwise the entire Lezíria region. Because the region experienced drainage and salinity problems collectors, drainage ditches and a defense dike where constructed. Being the region only 1-2 meters above the sea level, the construction of the dike made protection against tides and floods from the rivers Tejo and Sorraia possible. Because of these interventions “Lezíria Grande de Vila Franca de Xira” became a leveled, well drained area which doesn’t suffer influence from tides and floods (COTR, 2014; DGADR, 2014).

The irrigation water comes from the rivers Tejo and Sorraia entering the field’s trough openings in the defense dike. The water is directed to the agricultural parcels trough ditches that both irrigate and drain the soil. The outcome of this processes is somehow negative because affects water quality making the process of water management harder. The drainage of the Leziria’s soils is assured by exit canals mainly located in “Lezíria Sul”, which allows the water flow from the irrigation areas to the rivers Tejo and Sorraia (COTR, 2014).

1.1. Main crops

The soils present in "Lezíria de Vila Franca de Xira", are very fertile and show great agricultural potential for intensive irrigated practices, the crops used in the area have been changing through the years mainly due to the improvement of the water quality and its available amount, because of the opening of drainage ditches (ABLGVFX, 2014). The most cultivated crops during the period 2009-2014 were Tomato, Rice and Maize as shown in **Table II.1.**

Table II.1 – Evolution of the cultivated area in "Aproveitamento Hidroagrícola Lezíria Grande de Vila Franca de Xira (ABLGVFX, 2014).

Cultivated Crops "Aproveitamento Hidroagrícola Lezíria Grande de Vila Franca de Xira"	*2009*	*2010*	*2011*	*2012*	*2013*	*2014*
Tomato	1893	2307	2411	2216	2396	2769
Rice**	2553	3197	3742	3860	3826	3818
Maize	1599	1475	1791	2014	1856	1544
Sunflowers	259	90	174	20	196	130
Melon	164	135	173	134	117	149
Sorghum	157	277	180	206	56	41
Others	200	152	174	170	171	160
Fall-Winter Cereals (Irrigated) *	50	35	66	1012	-	-
Non Irrigated crops	507	641	379	79	1714	-
Crop Sum	7381	8310	9090	9711	8719	8610
Irrigated Crop Sum	6874	7669	8711	9632	8618	8610
Total usable area in Lezíria	12648	12648	12648	12648	12648	12648

*Area with irrigated Fall-Winter cereals with 2nd crops; **"Área de precários (Mouchão Lombo do Tejo)"

Figure II.1 shows the main crops in "Lezíria Grande" during Spring-Summer 2015, the most representative crops were Tomato and Rice followed by Maize.

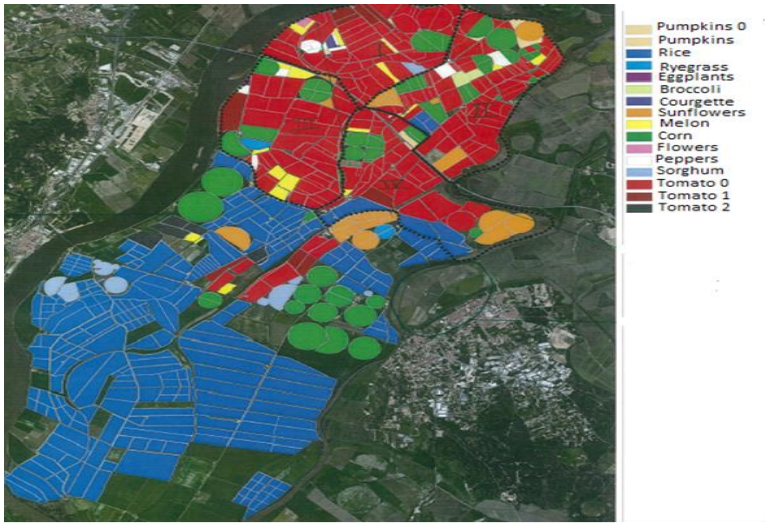


Figure II.1 – Main crops in "Lezíria Grande" Spring-Summer 2015 (ABLGVFX, 2015).

1.2. Brief analysis of the national tomato and maize production in 2013-14

Tomato

In the 2013-14 Agricultural year, tomato planting for the industry was held with no incidents, registering a raise in the cultivated area of 22,9% in comparison with last year, because of the stimuli promoted by the industry (extension of the reception period and improved contract terms) and favorable perspectives of exports to Spain (INE, 2015). The vegetative growth occurred naturally although the favorable weather conditions to the appearance of mildew and other fungi led to the increase of preventive treatments. The harvest was initiated in the 3rd week of July and went well until de 6th of September, when it started raining, and 30-35% of the cultivated area was still waiting to be harvested (INE, 2015). The access to harvest machines and mainly the transport vehicles for tomato became impossible, so the harvest could only be resumed in the last days of September. However, a significant part of the fruit was left on the ground (in between 10-20%) due to the impossibility of use for industrial processing. In conclusion the increase of the cultivated area was attenuated by the slight reduction in the unitary yield. In comparison with last year, production raised 20, 3%. The country imported in 2013 34 742 tons of tomato and exported 106 904 tons (INE, 2015).

As it can be seen on **Figure II.2** the surface occupied by tomato for industry was in 2012 13 895 ha, 14 006 in 2013 and 17 210 in 2014. The cultivated are in 2014 was significantly above the five-year average.

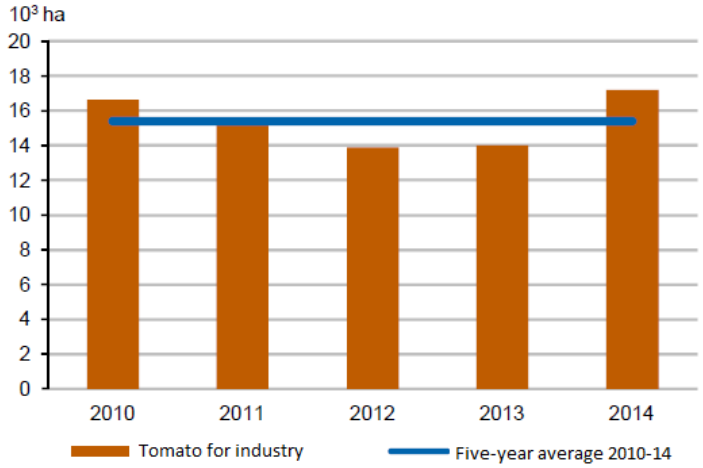


Figure II.2 – Tomato for industry area in Portugal (INE, 2015).

From the analysis of the **Figure II.3** we know that tomato for industry production in 2012 was 1 298 902 tons, in 2013 it decreased to 1 089 501 tons and in 2014 it raised to 1 310 366 tons. We

can say that in the last five years the production didn't oscillate much, recording its worst result in 2011 and 2013.

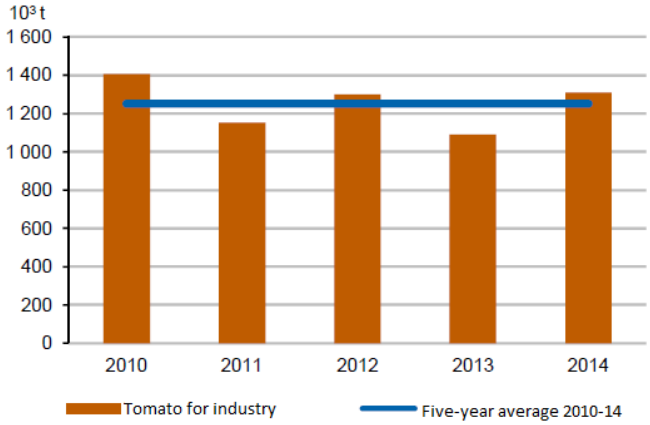


Figure II.3 - Tomato for industry production in Portugal (INE, 2015).

Maize

Cereals account for more than 60% of the world's agricultural production, being maize, rice and wheat the most produced (Pareja *et al*., 2011)

In the 2013-14 Agricultural year, the adverse climate conditions on early spring, namely the excess of soil moisture, forced the extension of maize sowings. On the other hand, the low temperatures delayed seed germination and the crop's initial growth (INE, 2015). After, the high moisture content difficult grain maturation, conditioning the harvest and raising the drying costs that allied with the price drop of this commodity on the international markets, contributed to a profitably decrease of corn culture (INE, 2015). Thus, maize surface and the respective registered production a slight decrease compared to 2013. Corn imports registered a value of 1 642 772 tons in 2013 and the exports value was only of 26 168, making the country very dependent of external markets. (INE, 2015).

As it can be seen on **Figure II.4** the surface occupied by maize in 2012 was 102 196 ha, in 2013 it raised significantly to 111 792 ha and in 2014 decreased to 107 642 ha. The years of 2013 and 2014 stood significantly above the five-year average.

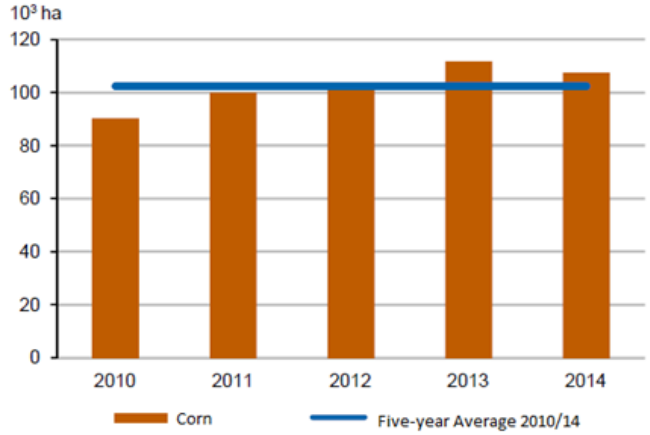


Figure II.4 – Maize area in Portugal 2010-14 (INE, 2015).

From the analysis of the **Figure II.5** we can see that Maize production in 2012 was 848 665 tons, in 2013 it raised to 929 538 tons and in 2014 it decreased again to 896 994 tons. Compared to the five-year average and especially to the year 2010 the corn production significantly raised in the country in the last years.

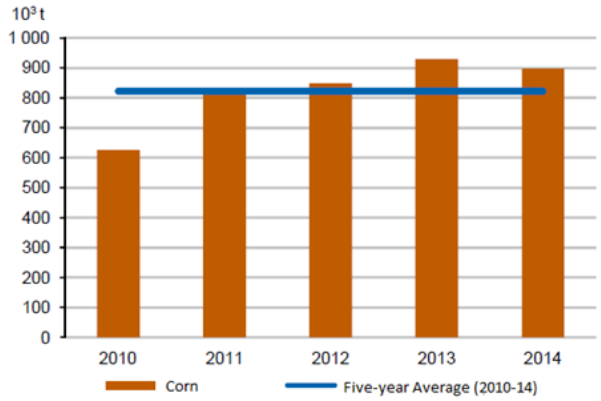


Figure II.5 – Maize Production in Portugal 2010-14 (INE, 2015).

1.3 Main diseases, pests and weeds in tomato and maize crops.

Tomato Pests

Pests and diseases are responsible for serious production losses during crop cycles, thus their knowledge and management are essential to minimize their impact. The most important pests in **tomato** for industry are shown on **Table II.2**.

Table II. 2 – Most important pests in tomato for industry crop in Portugal (Ibersem, 2011).

Scientific Name	Portuguese Common Name
Helicoverpa armígera	*"Lagarta do tomate"*
Aculops lycopersici	*"Ácaro do bronzeamento do tomateiro"*
Tuta absoluta	*" Traça do tomateiro"*
Liriomyza spp.	*"Lagarta mineira"*
Meloydogyne spp.	*"Nemátodos"*
Frankliniella occidentalis	*"Tripes" (as a vector of the TSWV)*
Aphis fabae; Macrosiphum euphorbiae	*"Afídeos"*

From these, the ones that account for more losses during the crop cycle according to (Mexia, A. 2014; Figueiredo, E. 2014) are:

Helicoverpa armigera (Hübner, 1805)*:*

This is the pest that in a direct and frequent form, causes major losses in tomato for industry crop. The caterpillar in the initial stages causes only minor damage because of feeding on leafs from tomato young shoots, later when it enters the fruits the losses are much bigger. Damage from this pest can cause production losses up to 15 tons/ha. To anticipate these losses, producers must monitor male flight curves, count and observe eggs and detect larvae in theirs first stages (L1 and L2) (Syngenta, 2016).

Aculops lycopersici (Tyron, 1917):

Losses arising from the attack of this mite are associated with plant inflorescences. Trough feeding this mite causes damage in the flower chalicesm fruit demorfation and irreversible deformation that tend to get bigger as the fruit grows. The attack on the leaves makes them look leathery promoting their early fall (Agrolynk, 2015)

Frankliniella occidentalis (Pergande, 1895)*:*

This pest causes indirect damage to the crop as a vector for the TSWV virus. Only larvae can acquire the virus and only the adults are responsible for the transmission. The virus leaves the plants with a tanned aspect and it's responsible for: spots or ring deformations in the fruits, necrotic spots on leaves, dwarfism, leaf rolling, general or marginal wilting among others causing major production losses (Mexia, A. 2014; Figueiredo, E. 2014).

Aphis fabae (Scopoli, 1763)*; Macrosiphum euphorbiae* (Thomas, 1878)*:*

These pests cause direct damages on the crops such as weakening and deformation of the plants, winding leaves, hyperplasia and falling of flower buds. They also cause indirect damage by virus transmission and accumulation of honeydew that causes reduction of photosynthesis and leaf burn (Mexia, A. 2014; Figueiredo, E. 2014).

Regarding diseases the most important in tomato for industry crop are present on the table II.3

Tomato diseases

Table II.3 – Most important diseases for tomato for industry crop in Portugal (Ibersem, 2011).

Scientific Name	Portuguese Common Name
Phythophtora infestans (Mont.) de Bary	*"Míldio"*
Alternaria alternate (Fr.) Keissl. (1912)	*"Alternariose"*
Pseudomonas syringae pv. Tomato (Van Hall, 1904)	*"Pinta bacteriana"*

Phythophtora infestans (Mont.) de Bary*:*

This disease affects all aerial parts of the tomato. The disease first manifests itself in the leaves, appearing large irregular patches of oily, dark green, which quickly acquire a brownish color and crunchy consistency. With continued wet weather, the board of these spots on the underside of the leaf may have a whitish color that is the vegetative body of the fungus - the mycelium. The leaf tissue affected gets a brownish color, wrinkles and ultimately die. With the evolution of the disease, petioles and stems are also affected in the same way, and if the bad weather conditions remain whole plant will die. In the unripe fruits this disease is manifested by brown spots which are generalized across the surface, these fruits subsequently suffer secondary infections with white mycelium and eventually rot (Syngenta, 2016).

Alternaria alternate (Fr.) Keissl. (1912):

This disease attacks all aerial parts of the plant during the entire crop cycle and can sometimes be a source of infection in the seedling production nursery. This disease usually does not cause damage leading to the destruction of the culture, however not controling it can lead to higher or lower production breaks and some qualitative depreciation of the fruits. The first signs of the presence of the disease are visible in the older leaves, manifesting itself by a collection of small dots of brown or black. The tissue surrounding the lesion may form a yellow halo. These necrotic spots rapidly increase in size and when they reach a diameter of about 6 mm can be distinguished concentric rings. The Alternaria also affects the inflorescences, which can lead to a high incidence of flower abortion, with obvious consequences on the production level. The presence of the disease in the final stages of tomato development can cause high defoliation and consequent scalds the fruit for its high exposure to the sun. Infection in green or ripe fruit occurs through their integration into the cup, the affected fruit present depressed concentric black spots which may affect a wide area (Syngenta, 2016).

*Pseudomonas syringae pv. Tomato (*Van Hall, 1904)*:*

Attacks all aerial parts of the plants. It is first observed on leaves in the form of small necrotic spots of brown color, usually surrounded by a yellow halo. The symptoms are more characteristic on the fruits, with formation of black dots on the surface, which can be torn off with a finger nail. The attack during flowering can cause a significant decrease on flower number (Embrapa, 2013).

Knowledge of adventitious flora of a crop, it's a key step in establishing a control strategy in the fight against weeds (Portugal, 2012). Besides identifying the species, their frequency and extent, environmental and cultural factors should also be known, as they decisively influence the flora. The floristic richness of the tomato crop for industry in different regions of the country round the hundred and fifty species (Portugal, 2012). However, the number of species that are more frequent and abundant simultaneously is relatively small not coming to two dozen (Portugal, 2012).

Table II.4: Main weeds affecting tomato crop in Portugal (Portugal, 2012)

Scientific Name	Portuguese Common Name
Amaranthus albus L.	"Bredo branco; Tristes"
Amaranthus retroflexus L.	"Moncos-de-Perú"
Chamaemelum mixtum (L.) All.	"Margaça"
Chenopodium album L.	"Catassol"
Convolvulus arvensis L.	"Corriola"
Cynodon dactylon (L.) Pers.	"Grama"
Cyperus spp.	"Junça"
Datura stramonium L.	"Figueira-do-inferno"
Digitaria sanguinalis (L.) Scop.	"Milhã digitada; Pé de galinha"
Echinochloa crus-galli (L.) P. Beauv	"Milhã pé de galo; Milhã vermelha"
Heliotropium europaeum L.	"Erva-das-verrugas"
Paspalum paspalodes (Michx) Scribner	"Graminhão"
Polygonum aviculare L.	"Sempre noiva; Sempre verde"
Polygonum lapathifolium L.	"Mal casada"
Portulaca olerácea L.	"Beldroega"
Raphanus raphanistrum L.	"Saramago"
Solanum nigrum L.	"Erva moura"
Sonchus oleraceus L.	"Serrallha macia"

Maize pests

According to Barros and Calado (2014) the most commom pests for maize crop in Portugal are: *Sesamia nonagrioides (*Lefèbvre, 1827*), Ostrinia nubilalis* (Hubner, 1796)*, Agrotis sp., Gryllotalpa gryllotalpa* (Linnaeus, 1758)*, Sitobion avenae* Fabricius*, A. metopolophium* (Walker, 1849)*, Rhopalosiphum maidis* (Fitch, 1856)*, Agriotes sp.*

Ostrinia nubilalis (Hubner, 1796): Tunnels made by the caterpillars cause disruption of stems and male flowers. The losses are considerable leading to production fall, plus harvesting is harder due to the damaged stems. The penetration holes favor the development of pathogens causing rot (Syngenta, 2014).

Agrotis sp.: Young caterpillar attacks lead to the devaluation of production or even the destruction of the crop (Syngenta, 2014).

Agriotes sp: Corn crop can be affected by early and late attacks. Damage represent some extensions of destruction; they can sometimes reach almost total destruction of the plot. The pins spend 80% of their life cycle in the larval buried in soil: we can find in the same field larvae of all ages measuring from 2 to 25 mm. Attracted by plants, the larvae move into the ground , stick or bite the underground part of the seedlings. They sometimes dig tunnels causing varying degrees of damage (Syngenta, 2014).

Maize diseases

According to Barros and Calado (2014) the most common diseases for maize crop in Portugal are: *Chephalosporium maydis, Cercospora zeae-maydis, Puccinia sorghi, Gibberella zeae, Colletotrichum graminícola, Exserohilum turcicum, Bipolaris maydis, G. moniliformis.*

Maize is very sensitive to weeds which compete for nutrients, space and light. According to Castelo (2013) the most common and particularly in Leziria's region are shown in **TableII.5.**

Table II.5: Main weeds affecting corn crop in Portugal

Scientific Name	Portuguese Common Name
Chenopodium ssp.	"Catassol"
Cynodon dactylon (L.) Pers., 1805	"Grama"
Cyperus spp.	"Junça"
Datura stramonium L.	"Figueira-do-inferno"
Digitaria sanguinallis (L.) Scop.	"Milhã-digitada"
Raphanus raphanistrum L.	"Saramago"

2. Pesticide characterization and selection

For the evaluation of pesticide effects on non-target zooplankton communities, fungicides, insecticides and herbicides were taken into consideration for their expected negative effects (either direct or indirect) to water invertebrates (Ippolito *et al*., 2015). Negative effects of insecticides and fungicides on invertebrates are presumably most often direct (with additional potential indirect effects on predators), whereas herbicides effects are partly caused by a decline of algae as a food resource (Ippolito *et al*., 2015).

The pesticides residues on the water that irrigates the sampling sites of the studied area for tomato and maize, were analyzed by an independent laboratory in 2014. This data is part of a broader research study in which the present studied is inserted and can be consulted in **ANNEX VI-X.**

Pesticides were selected based on their i) approval for use in the tomato and maize crops within the catchment of the river (**ANNEX I-V**), ii) actual use as indicated by farmers and the local associations (**ANNEX XXIII-XXIV)**; iii) physico-chemical characteristics and their consequent potential for surface water contamination (**ANNEX XI-XVI)**, being considered also some relevant metabolites resulting from them. So, it it's important to mention that not all applied pesticides were selected for the study. Among the pesticides that were found in the water analysis only the more toxic were selected (AMPA, glyphosate, metribuzin, imidacloprid, indoxacarb, chlorantrinaprole, cymoxanil, cypermethrin, metolachlor, desethyl-terbuthylazine and chlorpyrifos (-ethyl)).

3. Zooplankton sampling and identification

During the crop cycles of tomato and maize, six different sites of the irrigation canals were sampled for zooplankton from May to August 2014, tree in the tomato area (T1, T2 and T3), two in the maize area (M1 and M2) and one (R) in the irrigation canal upstream that served as a control site. The sampling procedure was carried out using a water-sampler with 1 L capacity that collected several depth-interated sub-samples until a 15 L samples were obtained; three replicates were collected for each one of the six sampling sites. The final samples were filtered through a 55 mm plankton net and immediately preserved in a 4% formalin solution. Water, pH, temperature, conductivity, and oxygen concentration were measured using a WTW Multiline F/set-3 multiprobe. In the ecotoxicology laboratory Rotifers, Copepods, Cladocerans and Ostracods were identified, counted and photographed with an Olympus CH-2 compound microscope using the Sedgewick-Rafter Cell method (APHA, 1992). From each one of the three replicates per collection date from all the sampling sites, 10 ml were analyzed divided in 10 Sedgewick-Rafter Cell observations, meaning that by the end of this work 510 individual observations of 1 ml were done. Rotifers were identified when possible to the species level and, Cladocerans to the family level, following Wongrant (2000). Ostracods and Copepods were only counted, being the last separated into nauplii, copepodite and adult stages. Rotifer eggs that were attached to the organisms were also registered.

CHAPTER III

A field-based approach to linking biological responses of zooplankton to freshwater contamination by pesticides.

Abstract

A study of zooplankton communities present in the irrigation water of maize and tomato crops under Portuguese Mediterranean conditions was carried out, to link possible relations between pesticide exposure and biological responses. This study is a contribution to the improvement of ERA of Pesticides under Mediterranian conditions. Six different sites of the irrigation canals were sampled for zooplankton during the crop cycles of tomato and maize (May to August 2014): tree in the tomato area (T1, T2 and T3), two in the maize area (M1 and M2) and one site in the irrigation canal upstream that served as a control site (R). A total of 37 rotifer species and 2 cladoceran families was identified. The main zooplankton group in all sampling sites was rotifer, as well as copepods in a nauplii stage that seem to be less affected by the pesticides. The concentration 12 ug/l of chlorpyrifos reduced the number of macrozooplankton, allowing the growth of rotifer densities. Values of 3.5-4.7 ug/l of chlorantrinaprole and 0.96 ug/l affect the size of the copepod community. Cladoceran and Ostracod comunnities decrease when glyphosate values are in the range of 2,3-3,9 ug/l. Values of glyphosate (0,66 ug/l), Ampa (0,88 ug/l) and Phosphate (2,38 mg/l) seem to be linked to lower Margalef index values (species richness).

Keywords: Pesticides, Zooplankton, Tomato and Maize crops, ERA, Ecology.

1. Introduction

Agroecosystems occupy almost one third of the worlds land area playing an important role in the maintenance of biological diversity. The study of biodiversity interrelated with agroecosystems is fundamental for biodiversity conservation since it plays an important role in agricultural production and ecologically sustainable agriculture (Bambaradeniya *et al*., 2004). Studies regarding the diversity and composition of zooplankton aiming as well as linking pesticide exposure to biological responses during crop cycles are still scarce in Portugal. The proportional risk associated with individual stressors, their cumulative effects and the way they interact to affect aquatic ecology is frequently unknown thus limiting the robustness of multiple-stressor ecological risk assessments (Preston, 2001). To learn more about the dynamics and recovery of zooplankton communities under Mediterranean conditions as well as their relation to natural and anthropogenic stressors, species richness, diversity and composition of these communities were examined, and pesticide residue levels monitored during a tomato and a corn crop cycle in “Leziria Grande de Vila Franca de Xira”. This work was carried out, aiming to

establish possible links between the effects in zooplankton ecology when exposed to pesticides during two different crop cycles (Tomato and Maize) under Mediterranean conditions. This study contributes for the improvement of the ecological relevance for the ERA of Pesticides in aquatic ecosystems.

2. Materials and Methods

2.1 Study Area

The present work was carried out in "Leziria Grande de Vila Franca de Xira", Central Portugal. This area receives water from the catchment of Conchoso (Tagus River), which is distributed through an irrigation canal (by water adduction) over the agricultural area. The field application system assures the transport of water within the fields and the drainage system removes the excess water (caused by rainfall and/or irrigation) from the fields. Study sites were selected in order to exclude the influence of waste-water treatment plants, industrial facilities, and mining drainage located upstream. Thus, chemical pollution other than from agricultural sources was unlikely. The selection of the sampling sites was also related to the type and distribution of tomato and maize crops and the drainage water bodies. Six sampling sites were selected, one reference/control site (R) in the irrigation canal upstream (to prevent influence from the selected crops areas application, three sampling sites in tomato production area (T1, T2,T3) and two sites in the maize production area (M1, M2).

2.2 Pesticides analysis

Water samples were collected in all sampling sites during 9 collecting campaigns in 2014 (May–August). Pesticides residues of chlorantrinaprole, chlorpyrifos, cymoxanil, cypermethrin,desethyl-terbuthylazine, imidacloprid, rimsulfuron and terbuthylazine were analysed by Liquid chromatography-tandem mass spectrometry (LC/MS/MS) with a LOQ of 0.05 µg/L, AMPA (Aminomethylphosphonic acid), glufosinat and glyphosate were analyzed trought derivatization/LC/MS/MS with a LOQ of 0.05 µg/L and chlorathalonil, chlorpyrifos (-ethyl), folpet metolachlor, metribuzin, lambda-cyhalothrin , indoxacarb and iprovilacarb were analyzed by SPE-GC/MS with a limit of quantification (LOQ) of 0.1 µg/L (DIN EN ISO/IEC 17025/2005).

2.3 Zooplankton Sampling

Zooplankcton biota was collected at all sampling sites using a water-sampler with 1 L capacity that collected several depth-interated sub-samples until a 15 L samples were obtained; three replicates were collected for each one of the six sampling sites. The final samples were filtered through a 55 mm plankton net and immediately preserved in a 4% formalin solution. Water, pH, temperature, conductivity, and oxygen concentration were measured using a WTW Multiline F/set-3 multiprobe. In the laboratory Rotifers, Copepods, Cladocerans and Ostracods were identified, quantified and photographed with an Olympus CH-2 compound microscope using the Sedgewick-Rafter Cell method (APHA, 1992). Rotifers were identified when possible to the species level, Cladocerans to the family level, following Wongrant (2000). Ostracods and Copepods were only quantified, being the last separated into nauplii, copepodite and adult stages. Rotifer eggs that were attached to the organisms were also registered.

2.4. Data treatment

Zooplankton Groups' and Total abundance, Diversity were identified using Shannon-Wiener index (H'), Pielou index (J) and Margalef's richness index. Firstly, aiming at fitting a multiple regression model which would allow the prediction of changes in species abundance, diversity, richness, and evenness. Possible relationships with explanatory variables and their interactions were attained. Secondly, to try to establish possible relations between response and explanatory variables, a multivariate analysis was performed using the ordination method RDA with the aid of the specialized multivariate analysis program CANOCO – Canonical Community Ordination (ter Braak and Smilauer 1998). The environmental data was separated in, 6 species (Sp) and 3 environmental (Env) matrixes. Zooplankton groups in tomato sampling sites (Sp1), Zooplankton groups in maize sampling sites (Sp2), Rotifers species in tomato sampling sites (Sp3), Rotifers species in maize sampling sites (Sp4), Rotifer species in the R site (Sp5) and Zooplankton groups in the R site (Sp6), Pesticide and environmental parameters in tomato sampling sites (Env1), Pesticide and environmental parameters in maize sampling sites (Env2) and Pesticides in R sampling site (Env3).

The following table shows the main environmental variables that are present in the environmental matrixes used in this study.

Table III.1: Environmental variables present in the analysis of Env1, Env2 and Env3 environmental matrixes.

Environmental variables	Env1	Env2	Env3
AMPA	X	X	X
glyphosate	X	X	X
metribuzin	X		
imidacloprid	X		
indoxacarb	X		
chlorantrinaprole	X		
cymoxanil	X		
cypermethrin		X	
metolachlor		X	X
terbuthylazine		X	X
desethyl-terbuthylazine		X	
chlorpyrifos (-ethyl)		X	
NH4+-N (mg L-1)	X	X	
P (mg L-1)	X	X	
NO3--N (mg L-1)	X	X	
ChlorA	X	X	
Water temperature (ºC)	X	X	
pH	X	X	
Conductivity	X	X	
O2 mg/l	X	X	
O2 %	X	X	
Alkalinity	X	X	
Shanon index	X	X	X
Pielou index	X	X	X
Margalef's index	X	X	X

A detrended correspondence analysis (DCA) was applied to all the species matrixes in order to evaluate the best fitting model to be applied. The resulting values for the length of gradient in the first axis for all six species matrixes were lower than 3 (limit adopted by CANOCO), revealing that a Redundancy analysis (RDA) was the best-chosen method for the treatment of theenvironmental data. The following species x environmental matrixes combinations were analysed using the RDA: Sp1 x Env1, Sp2 x Env2, Sp3 x Env3, Sp4 x Env2, Sp5 x Env3 and Sp6 x Env3. While performing the RDA's the Varience inflation factor (VIF) of each environmental variable (pesticides and environmental parameters) was analyzed to exclude collinearity between variables with VIF > 20 that implies collinearity.

3. Results:

3.1. Environmental parameters

From the three sampling sites of the tomato crop, Tomato sampling site one (T1) showed higher mean values for all the variables listed on the Table (III.2).

Table III.2: Mean values of environmental variables in tomato sampling sites (T1, T2, T3) along time.

Environmental Variables		19th May	30th May	6th Jun	16th Jun	26th Jun	7th Jul	17th Jul	28th Jul	7th Aug	Mean
Water temperature (ºC)	T1	22.1	19.1	21	23.2	20.5	21.6	21.8	18.4	20.6	20.92
	T2	22.5	20.3	22	24.1	21	22	-	19.4	22	19.26
	T3	24	20.7	22.3	25	21	21.5	-	21	22	19.72
pH	T1	7.11	7.27	6.77	6.85	7.14	7.13	7.04	6.79	6.18	6.92
	T2	6.77	6.85	7.11	7.27	7.14	7.13	-	6.79	6.18	6.14
	T3	7.27	7.11	6.77	7.38	7.14	7.13	-	6.18	6.79	6.20
Conductivity (uS/cm)	T1	359	255	345	204	255	2.54	350	223	200	243.73
	T2	191	204	300	359	255	2.54	-	223	200	192.73
	T3	359	200	205	359	255	2.54	-	200	223	200.39
O2 mg/l	T1	7.27	6.14	5.36	4.13	5.82	5.82	6.01	5.14	6	5.74
	T2	5.36	4.13	7.27	6.14	5.82	5.82	-	5.14	6	5.076
	T3	6.14	7.27	5.36	6.14	5.82	5.82	-	6	5.14	5.30
O2 %	T1	81.8	74.5	60.8	45.6	64.5	64.5	76.2	59	71	66.43
	T2	60.8	45.6	81.8	74.5	64.5	64.5	-	59	71	57.97
	T3	74.5	81.8	60.8	74.5	64.5	64.5	-	71	59	61.18
Alkalinity	T1	30	105	78	64	98	98	206	86	80	93.89
	T2	78	64	30	105	98	98	-	86	80	71
	T3	105	30	78	105	98	98	-	80	86	75.56
Chlorophyll a (ug/L)	T1	16.8	10.1	9.1	5.8	4.6	9.1	5.3	4.3	6.4	7.944
	T2	15	10.1	8.9	5.6	4	9.1	-	4	5.8	6.944
	T3	16	11	9	5.7	4.5	9.2	-	4.2	6.4	7.33
NH4+-N (mg L-1)	T1	2.12	2.80	2.12	3.69	5.75	4.48	5.04	6.78	4.05	4.09
	T2	0.55	0.59	0.77	0.34	0.43	1.17	-	2.06	2.06	0.89
	T3	0.55	1.00	1.61	0.15	3.12	4.61	-	4.77	2.90	2.08
P (mg L-1)	T1	0.75	0.85	0.27	0.30	0.51	0.20	0.75	1.37	1.00	0.67
	T2	0	0.00	0.00	0.00	0.00	0.02	-	0.02	0.02	0.0067
	T3	0	0.00	0.00	0.00	0.00	0.00	-	0.00	0.01	0.0011
NO3--N (mg L-1)	T1	0	0.11	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.02
	T2	0	0.08	0.00	0.06	0.00	0.00	-	0.00	0.00	0.0156
	T3	0.05	0.21	0.05	0.00	0.00	0.00	-	0.00	0.00	0.034

In the two maize crop sampling sites (M1, M2), M2 showed higher mean values for all variables except for chlorophyll a, where the average value was 7.68 for both M1 and M2 as shown on table (III.3)

Table III.3: Mean values of environmental variables in maize sampling sites (M1, M2) along time.

Environmental Variables		19th May	30th May	6th Jun	16th Jun	26th Jun	7th Jul	17th Jul	28th Jul	7th Aug	Mean
Water temperature (ºC)	M1	20.3	19.8	21.5	25.1	20.3	21	22	20.3	21	21.26
	M2	22	20.3	21	25.6	20.3	21	21.5	19.8	20.3	21.31
pH	M1	6.85	7.25	6.77	7.27	6.85	7.14	6.79	6.85	7.13	6.99
	M2	6.79	6.85	7.14	8	6.85	7.13	6.77	7.25	6.85	7.07
Conductivity (uS/cm)	M1	204	270	191	359	204	255	223	210	2.54	213.17
	M2	223	204	255	401	210	2.54	191	270	204	217.84
O2 mg/l	M1	4.13	4.89	5.36	6.14	4.13	5.82	5.14	6	5.82	5.27
	M2	5.14	4.13	5.82	7.89	6	5.82	5.36	4.89	4.13	5.46
O2 %	M1	45.6	53.3	60.8	74.5	45.6	64.5	59	74.5	64.5	60.26
	M2	59	45.6	64.5	89.8	74.5	64.5	60.8	53.3	45.6	61.96
Alkalinity	M1	64	116	78	105	64	98	86	105	98	90.44
	M2	86	64	98	110	105	98	78	116	64	91
Chlorophyll a (ug/L)	M1	15.7	9.9	9	6	4.2	8.9	5.2	4.3	5.9	7.68
	M2	16	10.1	9.1	5.4	4.1	9	5.1	3.9	6.4	7.68
NH4+-N (mg L-1)	M1	0.01	0.25	0.99	0.63	1.07	1.03	0.89	0.51	1.60	0.78
	M2	0.02	0.38	0.71	1.57	2.21	2.65	5.83	4.66	4.67	2.52
P (mg L-1)	M1	0.00	1.23	0.66	0.17	2.86	2.13	2.70	2.56	2.38	1.63
	M2	0	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.002
NO3--N (mg L-1)	M1	0	0.05	0.00	0.00	2.31	2.13	0.55	0.00	0.04	0.56
	M2	0.00	0.02	0.00	4.66	0.05	0.05	0.02	0.00	0.40	0.58

3.2. Zooplankton Total Abundance

In both tomato (T1, T2, T3) and maize (M1, M2) crops, as well in the control site (R) a total of 37 different species of rotifers were observed as shown on **Table III.4 and Figures III.1 -11.** In the maize M1 sampling site 19 rotifer species were registered. The highest total abundance (2528) was registered for *Brachionus rotundiformis* (Tschugunnoff, 1921) and the lowest (17) for *Cephalodella gibba* (Ehrenberg, 1830) as shown in Annex XXXIII. While in the second maize sampling site (M2) only 4 species were registered. The highest total abundance (53938) for *Brachionus rotundiformis* (Tschugunnoff, 1921) and the lowest (37) for the species Filinia brachiata (Rousselet, 1901) as shown in Annex XXXVI. In the tomato sampling site T1 were registed 17 rotifer species. The highest total abundance (2708) was registed for *M. ventralis sp*. and the lowest (7) for Filinia cornuta sp. as shown in Annex XXII. In T2, 20 rotifer species were

observed. The highest count (238) corresponded to *L. patella* (O.F. Müller, 1786) and the lowest (2) to *Filinia cornuta sp* as shown in Annexx XXVI. In the T3 sampling site, 20 rotifer species were observed. The highest total abundance (4008) belonged to *L. patella* (O.F. Müller, 1786) and the lowest (3) to *Platyias quadricornis* (Ehrenberg, 1832) as shown in Annex XXIX. In the control sampling site (R) 24 rotifer species were observed. The highest total abundance of organisms most numerous was *Brachionus rotundiformis* (Tschugunnoff, 1921) with 7725 organisms and the lowest (3) were the species: *Hexartha sp*., *Filinia brachiata* (Rousselet, 1901) and *M. ventralis* sp. as shown in Annex XXXIX. Regarding Ostracods, the total abundance values: T1 (888), T2 (368), T3 (122), M1 (248), M2 (45) and R (11) as shown in Annexes XXIV, XXVIII, XXXI, XXXV, XXXVIII and XXXXI. Regarding Cladocera, 2 Families were observed (Chydoridae and Moinidae), the total abundance values were: T1 (9 for Chydoridae), T3 (3 for Chydoridae) and R (67 for Moinidae) as shown in Annexes XXV, XXXII and XXXXII. Finally regarding Copepods total abundance values for the 3 stages (nauplius, copepodite and adult) in all sampling sites were: T1 (377 for nauplius, 186 for copepodite and 137 for adult), T2 (282 for nauplius and 6 for copepodite), T3 (1050 for nauplius, 95 for copepodite and 58 for adult), M1 (14085 for nauplius, 250 for copepodite and 29 for adult), M2 (783 for nauplius) and R (395 for nauplius, 16 for copepodite and 2 for adult) as shown in Annexes XXIII, XXVII, XXX, XXXIV, XXXVII and XXXX respectively.

The following table shows a list of the observed rotifer species in all the sampling sites: maize (M1, M2); tomato (T1,T2,T3) and control (R).

Table III.4: List of observed rotifer species in all sampling sites: maize (M1,M2); tomato (T1,T2) and control (R).

Family	Species	M1	M2	T1	T2	T3	R
Brachionidae	*Brachionus calyciflorus* (Pallas, 1766)			X	X	X	X
	Brachionus rotundiformis (Tschugunoff, 1921)	X	X	X	X	X	X
	Brachionus angularis (Gosse, 1851)	X		X	X		X
	Brachionus urceolaris (O.F. Müller, 1773)	X				X	X
	Brachionus quadridentatus (Hermann, 1783)	X			X	X	X
	Platyias quadricornis (Ehrenberg, 1832)			X		X	
	Keratella tropica sp.			X			X
	Keratella cochlearis (Gosse, 1851)						X
Lecanidae	*L. quadridentata* (Ehrenberg, 1830)			X	X	X	X
	Lecane sp1			X		X	
	Lecane sp2	X		X	X	X	
	Lecane sp3			X	X	X	
	Lecane sp4			X	X		
Lepadellidae	*Colurella sp.*	X			X	X	
	L. patella (O.F. Müller, 1786)	X		X	X	X	
Mytilinidae	*M. mucronata var macracantha* (Gosse, 1886)	X					
	M. ventralis sp.			X	X	X	
Trochosphaeridae	*Filinia brachiata* (Rousselet, 1901)	X	X		X	X	X
	Filinia cornuta sp.			X	X		X
	Filinia terminalis (Plate, 1886)						X
Hexarthridae	*Hexarthra sp.*						X
Gastropodidae	*Ascomorpha sp.*	X					X
Notommatidae	*Cephalodella gibba* (Ehrenberg, 1830)	X					
	Cephalodella forficula (Ehrenberg, 1832)						X
Synchaetidae	*Synchaeta sp.*	X					X
	Polyartha sp.				X	X	X
Trichocercidae	*Trichocerca sp.*	X	X		X	X	X
Other rotifers	*Rsp1*	X	X	X	X	X	X
	Rsp2	X			X	X	X
	Rsp3	X		X			
	Rsp4			X	X	X	X
	Rsp5	X			X	X	X
	Rsp6			X	X	X	X
	Rsp7	X					
	Rsp8	X					
	Rsp9						X
	Rsp10						X

Rsp - organisms that could not be identified to any taxonomical level (10) were described with the code Rsp followed by a number from 1-10.

The following presented images represent all the rotifer species that were observed during this study:

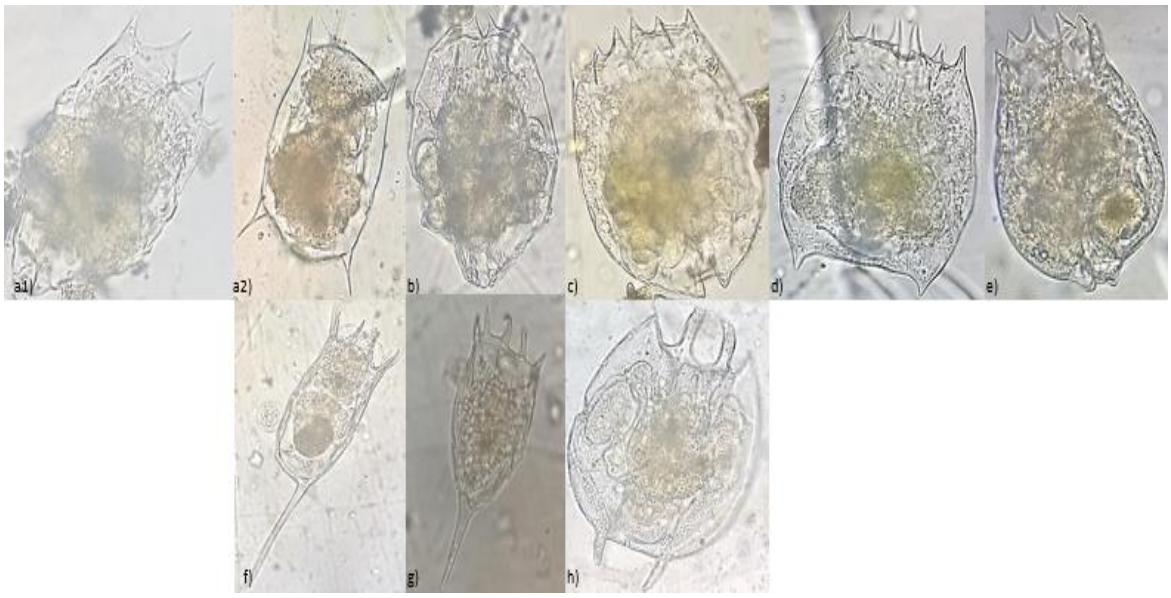


Figure III.1: Rotifers of the Brachionidae Family a1) *Brachionus calyciflorus (Pallas, 1766)* Polymorphism 1; a2) Brachionus calyciflorus (Pallas, 1766) Polymorphism 2; b) *Brachionus angularis (Gosse, 1851); c) Brachionus urceolaris (O.F. Müller, 1773); d) Brachionus quadridentatus (Hermann, 1783); e) Brachionus rotundiformis (Tschugunoff, 1921); f) Keratella tropica sp; g) Keratella cochlearis (Gosse, 1851); h) Platyias quadricornis (Ehrenberg, 1832),* (Oliveira,2015).

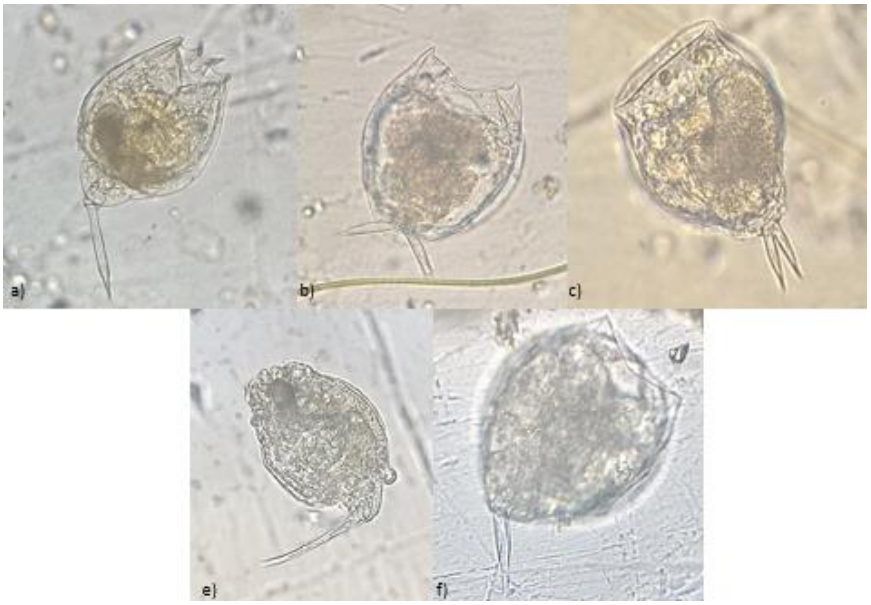


Figure III.2: Rotifers of the Lecanidae Family a) *L. quadridentata (Ehrenberg, 1830); b) Lecane sp1; c) Lecane sp2; e) Lecane sp3; f) Lecane sp4,* (Oliveira, 2015).

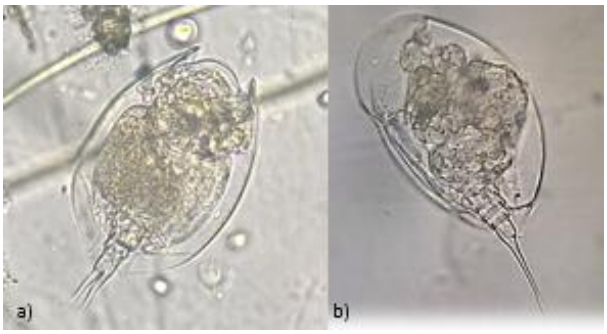


Figure III.3: Rotifers of the Family Lepadellidae a) *L. patella (O.F. Müller, 1786); b) Colurella sp*. (Oliveira, 2015).

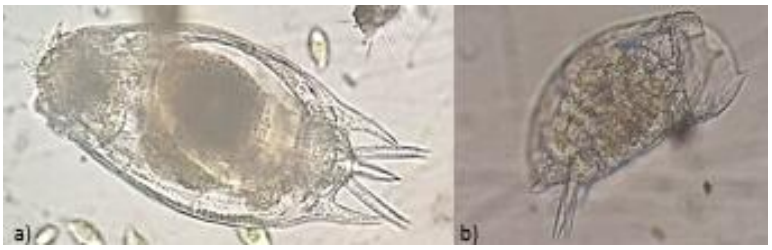


Figure III.4: Rotifers of the Family Mytilinidae a) *M. mucronata var macracantha (Gosse, 1886); b) M. Ventralis sp.* (Oliveira, 2015).

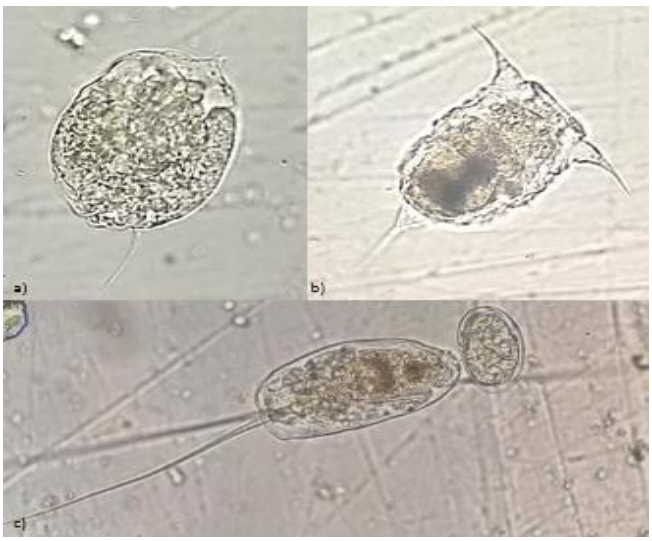


Figure III.5: Rotifers if the Family Trochosphaeridae *a) Filinia cornuta sp.; b) Filinia brachiata (Rousselet, 1901); c) Filinia terminalis (Plate, 1886),* (Oliveira,2015).

Figure III.6: Rotifer of the Family Hexarthridae a) *Hexarthra sp.* (Oliveira, 2015).

Figure III.7: Rotifer of the Family Gastropodidae a) *Ascomorpha sp.* (Oliveira, 2015).

Figure III.8: Rotifers of the Family Notommatidae *a) Cephalodella gibba (Ehrenberg, 1830); b) Cephalodella forficula (Ehrenberg, 1832)* (Oliveira, 2015).

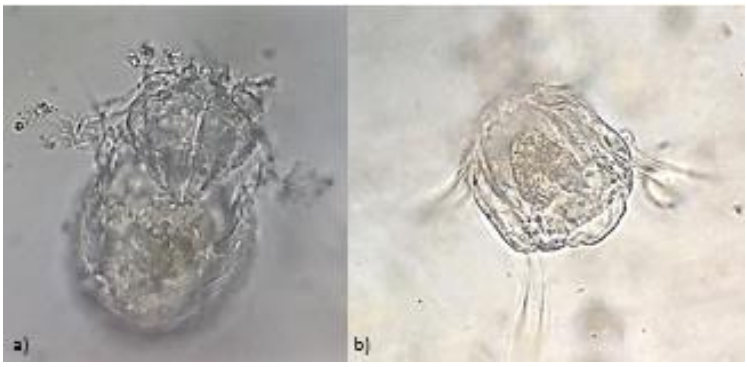


Figure III.9: Rotifers of the Family Synchaetidae a) *Synchaeta sp.; b) Polyartha sp.* (Oliveira, 2015).

Figure III.10: Rotifer of the Family Trichocercidae *a) Trichocerca sp.* (Oliveira, 2015).

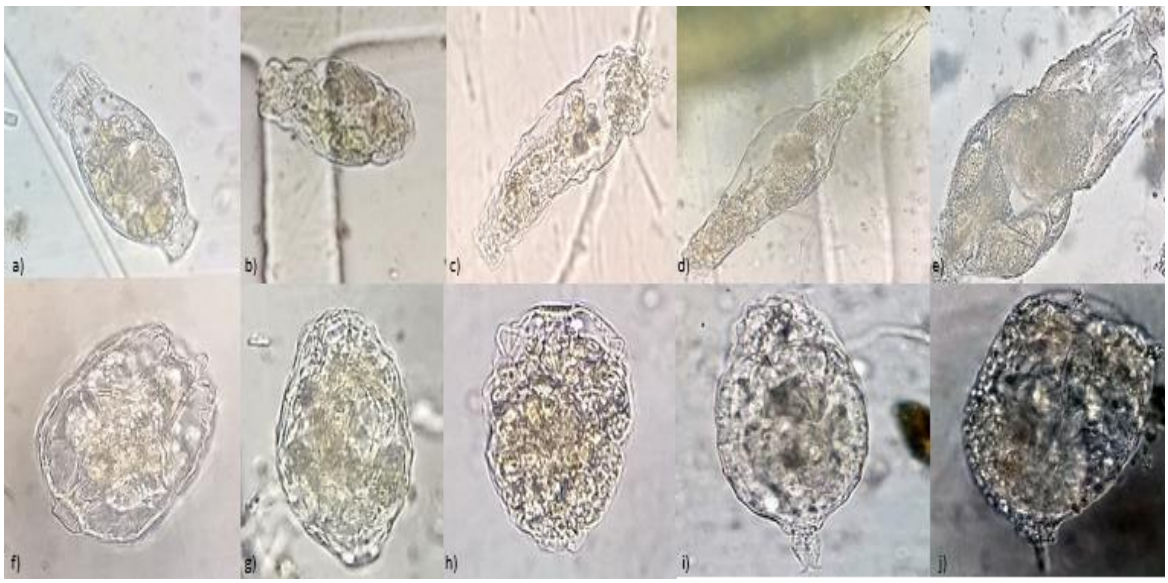


Figure III.11: Other rotifer a) Rsp1; b) Rsp2; c) Rsp3; d) Rsp4; e) Rsp5; f) Rsp6; g) Rsp7; h) Rsp8; i) Rsp9; j) Rsp10, (Oliveira, 2015).

Eggs that were attached to rotifers were counted and the results can be consulted on **Table III.5.** The sampling sites with more rotifer eggs were on maize crop (M2) and in the control site (R).

Table III.5: Rotifer egg count in all sampling sites: maize (M1, M2); tomato (T1,T2) and control (R). along time.

Site	*19th May*	*30th May*	*06th Jun*	*16th Jun*	*26th Jun*	*07th Jul*	*17th Jul*	*28th Jul*	*07th Aug*
T1	0	2^{e} + 12^{a} +7^{b}	3^{b}	0	3^{a} + 4^{b}	9^{a}	0	0	0
T2	0	0	0	0	8^{c}	3^{e} + 1^{b} + 26^{c}	-	-	0
T3	0	0	4^{e}+ 3^{f}	2^{f}	0	1^{e} + 3^{a} + 8^{c}	0	0	0
Total T	0	21	10	2	15	51	0	0	0
M1	13^{a} + 9^{d}	0	11^{a}	40^{a}	0	0	2^{a}	3^{a} + 2^{b}	3^{c}
M2	1183^{a}	288^{a}	1033^{a}	5923^{a}	7551^{a}	8933^{a}	527^{a}	-	283^{a} + 16^{c}
Total M	1205	288	1044	5963	7551	8933	529	5	302
R	2^{e}	101^{a} + 27^{e} + 36^{b} + 8^{h}	20^{b} + 11^{h} + 9^{j}	0	80^{a} + 6^{k} + 1^{g}	680^{a} + 1^{j}	3^{a}	0	13^{a} + 2^{e} + 3^{i}
Total (T + M +R)	1207	481	1094	5965	7653	9665	532	5	320

a - *Brachionus rotundiformis (Tschugunoff, 1921); b - Brachionus angularis (Gosse, 1851); c - Filinia brachiata (Rousselet, 1901); d – Rsp8; e - Brachionus calyciflorus (Pallas, 1766); f - Brachionus urceolaris (O.F. Müller, 1773); g - Brachionus quadridentatus (Hermann, 1783); h - Filinia terminalis (Plate, 1886); i - Filinia cornuta sp.; j - Keratella tropica sp; k - Polyartha sp.;* - No water available for sampling.

3.3 Zooplankton densities in water

Zooplankton abundance regarding the tomato crop in the three sampling sites along time is shown in Annex I. The highest mean densities for three replicas of each sampling site (maize (M1, M2), tomato (T1, T2, T3) and control (R)) registered were 1114.6 ind.L^{-1}, 735.8 ind.L^{-1} and 258.7 ind.L^{-1} in T1, T3 and T2 sampling sites respectively, being Rotifers the most representative group in all sites. The total abundance values for the maize crop in the two sampling sites along time are shown in Annex II. The highest mean densities registered were 7004.9 ind. L^{-1} and 2399 ind. L^{-1} for the M2 and M1 sampling sites respectively, Rotifers were the most abundant organisms. The mean density for the tomato and maize crop was 713 ind. L^{-1} and 4701.9 ind. L^{-1} respectively. For the control (R) sampling site, values of total abundance along time are shown on Annex III. The mean density value was 1175.6 ind. L^{-1}, being Rotifers the most abundant organisms.

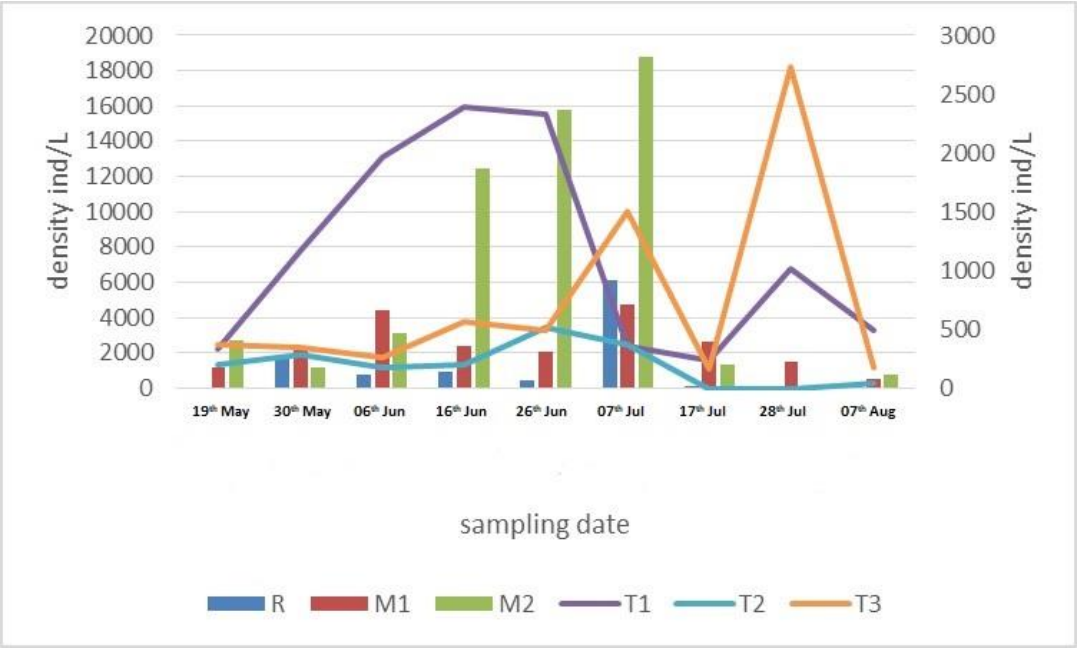


Figure III.12: Mean densities in all sampling sites: maize (M1,M2); tomato (T1,T2) and control (R) along time.

3.4. Zooplankton diversity

Three indexes: Shannon diversity index, Pielou evenness index and Margalef species richness index, were calculated in all sampling sites (maize: (M1,M2), tomato: (T1,T2,T3) and control: (R) and are shown on Table III.6.

Table III.6: Shannn, Pielou and Margalef indexes in all sampling sites: maize (M1,M2); tomato (T1,T2) and control (R), along time.

	Tomato Sampling Sites									Maize Sampling Sites						Control		
	T1			T2			T3			M1			M2			R		
Date	H'	J	DM	H'	J	DM	H'	J	DM	H'	J	DM	H'	J	DM	H'	J	DM
19th May	0.90	**0.39**	1.55	0.77	0.34	1.70	0.34	0.15	1.52	0.69	0.31	1.13	0.09	0.06	0.51	0.57	**0.32**	1.23
30th May	0.72	0.29	1.56	0.73	0.29	1.94	0.83	0.36	1.54	0.58	0.27	1.04	**0.39**	0.24	**0.57**	0.12	0.05	1.73
06th Jun	**0.95**	0.37	**1.58**	0.79	0.29	2.88	**0.97**	**0.39**	1.98	0.72	0.29	1.31	0.34	**0.21**	0.50	**0.60**	0.23	1.96
16th Jun	0.30	0.13	1.16	0.81	0.29	**3.01**	0.50	0.18	**2.37**	0.53	0.27	0.77	0.03	0.02	0.42	0.44	0.19	1.46
26th Jun	0.28	0.12	1.29	0.83	0.31	2.08	0.45	0.19	1.45	0.65	0.33	0.79	0.04	0.02	0.41	0.30	0.13	1.46
07th Jul	0.17	0.08	1.36	**0.92**	**0.37**	1.85	0.33	0.13	1.64	0.21	0.12	0.59	0.04	0.04	0.20	0.08	0.04	0.69
17th Jul	0.18	0.08	1.66				0.55	0.34	0.79	0.29	0.14	0.89	0.09	0.06	0.56	0.31	0.16	1.25
28th Jul	0.06	0.03	1.01				0.39	0.18	1.01	0.67	0.29	1.23				0.37	0.16	**2.09**
07th Aug	0.12	0.06	0.81	0.77	**0.37**	1.92	0.55	0.26	1.34	**0.80**	**0.35**	**1.44**				0.14	0.06	1.72

Note: Shannon diversity index (H'); Pielou evenness index (J); Margalef species richness index (DM)

The highest Shannon diversity index values in tomato sampling sites (T1,T2 and T3) were: T3 sampling site (H'=0.97) followed by T1 (H'=0.95) and T2 (H'=0.92). The highest Evenness (J) value was registered in the T3 sampling site (J=0.39) equal to T1 (J=0.39) and by T2 (J=0.37). Regarding Margalef's Diversity index the highest value was registered in the T2 sampling site ($D_M = 3.01$)

followed by T3 (D_M *=2,37) and T1 (D_M =1.58).* Regarding the two maize crop sampling sites (M1 and M2) he highest Shannon diversity index values in each sampling site were: M1 sampling site (H'=0.80) followed by M2 (H'=0.39). The highest Evenness (J) value was registered in the M1 sampling site (J=0.35) followed by M2 (J=0.21). Regarding Margalef's Diversity index the highest value was registered in the M1 sampling site (D_M *= 1.44*) followed by M2 (D_M *=0.57).* Regarding the control sampling site (R) the Shannon diversity index value was (H'=0.60), the Evenness value was (J=0.32) and the Margalef's diversity index value was (D_M *=2,09).*

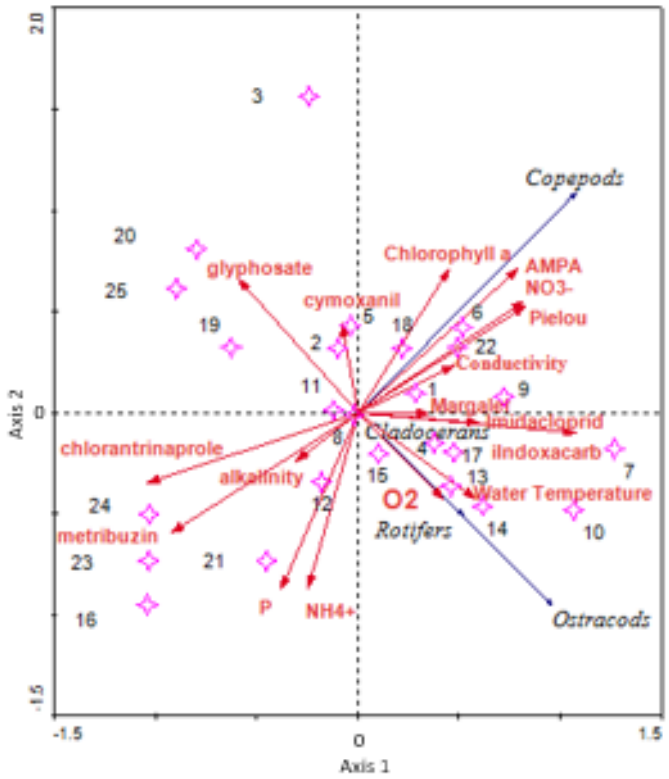
3.5 Statistical analysis:

Significant interactions between zooplankton diversity and richness of both crops and explanatory variables (AMPA, glyphosate, metribuzin, imidacloprid, indoxacarb, chlorantrinaprole, cymoxanil, cypermethrin, metolachlor, terbuthylazine, desethyl-terbuthylazine, chlorpyrifos (-ethyl)) and their interactions were found using the multiple regression model. However a significant association within the maize crop (negative correlation), between the three calculated diversity indexes and the insecticide chlorpyrifos (Shannon: -0,3150; Pielou: -0,3216; Margalef: -0,2355) was identified due to a single sample (19th May) that had a very high residue (12 ug/l confirmer) value for this pesticide. Regarding the RDA analysis, only 3 species x environmental matrixes from a total of 6 were selected (sp1 x env1; sp2 x env2 and sp6 x env3) because these were the ones where the environmental variables explained the zooplankton variation more of the total variation in the Axis 1 and 2. In the tomato crop (sp1 x env 1), the RDA accepted model revealed 15 environmental variables influencing the zooplankton communities in all tomato sampling sites (Table III.6). Indoxacarb is the variable more correlated with the Axis 1. This axis has 41% total explanatory percentage.

Table III.7: Correlations of the environmental variables with the canonical axes from sp1 x env1 RDA; influence as a percentage of total variation for the corresponding axis; stronger correlations in bold.

Environmental variables	Correlation with canonical axes	
	Axis 1 (41%)	Axis 2 (29,3 %)
AMPA	0.29	**0.31**
glyphosate	-0.22	**0.28**
metribuzin	**-0.34**	-0.25
imidacloprid	0.22	-0.02
indoxacarb	**0.40**	-0.04
chlorantrinaprole	**-0.38**	-0.15
cymoxanil	-0.03	0.19
NH4+-N (mg L-1)	-0.09	**-0.37**
P (mg L-1)	-0.14	**-0.37**
NO3--N (mg L-1)	**0.30**	0.24
Chlorophyll a (ug/L)	0.17	**0.30**
Water temperature (ºC)	**0.21**	-0.18
conductivity	**0.18**	0.1
O2	0.15	**-0.18**
Alkalinity	**-0.11**	-0.10
Pielou	**0.31**	0.23
Margalef	**0.13**	-0.002

Regarding the tomato RDA triplot, the dates: 23 – 07th Aug of T1 sampling site and 24 – 07th Aug of the T2 sampling site show that higher values of chlorantrinaprole and metribuzin are negatively correlated to the number of Copepods and the Pielou Index. The dates: 19 – 17th Jul of the T1 sampling site and 20 – 17th Jul, 25 – 07th Aug of the T3 sampling site, show that high glyphosate values are negatively correlated with the number of Rotifers, Cladocerans and Ostracods.



Dates T1: **1** - 19th May, **4** – 30th May, **7** – 06th Jun , **10** – 16th Jun, **13** – 26th Jun, **16** – 07th Jul, **19** – 17th Jul, **21** – 28th Jul **23** – 07th Aug; **Dates T2: 2** – 19th May, **5** – 30th May, **8** – 06th Jun, **11** – 16th Jun, **14** – 26th Jun, **17** – 07th Jul , **24** – 07th Aug ; **Dates T3: 3** – 19th May, **6** – 30th May, **9** – 06th Jun, **12** – 16th Jun, **15** – 26th Jun, **18** – 07th Jul, **20** – 17th Jul, **22** – 28th Jul, **25** – 07th Aug.

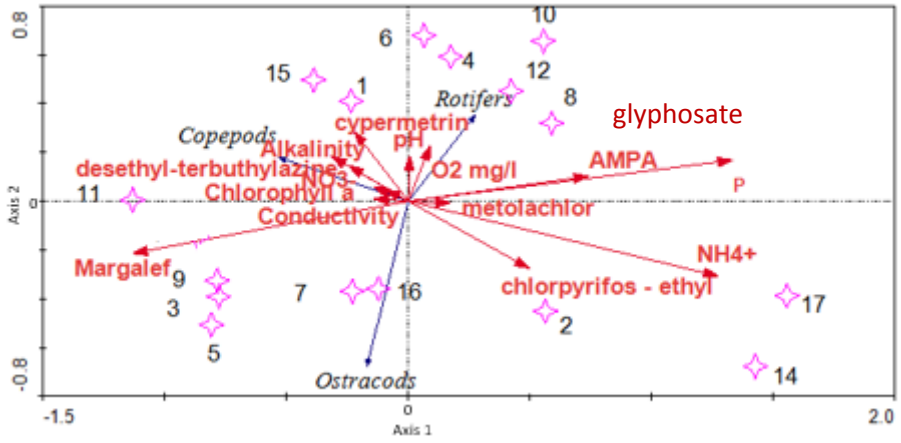
Figure III.13: RDA plot of zooplankton groups and environmental variables in the three tomato sampling sites (T1,T2 and T3).

In maize (sp2 x env 2), the RDA accepted model revealed 14 environmental variables influencing the zooplankton communities in all maize sampling sites (Table III.7). Phosphate is the variable more correlated with the Axis 1. This axis explains 57,8% of total variation.

Table III.8: Correlations of the environmental variables with the canonical axes from sp2 x env2 RDA; influence as a percentage of total variation for the corresponding axis; stronger correlations in bold.

Environmental variables	Correlation with canonical axes	
	Axis 1 (57,8%)	Axis 2 (21,1 %)
AMPA	**0.42**	0.08
cypermethrin	-0.13	**0.23**
metolachlor	**0.1**	-0.005
desethyl-terbuthylazine	**-0.14**	0.12
chlorpyrifos (-ethyl)	**0.28**	-0.22
Ph	0.003	**0.15**
Conductivity	**-0.08**	0.008
O2 mg/l	0.05	**0.18**
Alcalinity	**-0.18**	0.16
Chlorophyll a (ug/L)	**-0.06**	0.04
NH4+-N (mg L-1)	**0.73**	-0.25
P (mg L-1)	**0.76**	0.14
NO3--N (mg L-1)	**-0.08**	0.05
Margalef	**-0.65**	-0.17

Regarding the Maize RDA triplot, higher values of glyphosate, Ampa and P (Phosphate) seem to be linked to lower Margalef index values in the dates: **3** – 30th May, **5** – 06th Jun, **9** – 26th Jun and 11 – 07th Jul of the M1 sampling site, since Margalef index vector is diametrically opposed to the vectors of glyphosate , Ampa, Phosphate and $Nh4^{+}$. Copepods on the dates: **2** - 19th May, **17** – 07th Aug of the M2 sampling site and on **14** – 17th Jul of the M1 sampling site indicate to ne negatively affected by the chlorpyrifos – ethyl.



Dates M1: 1 – 19th May, **3** – 30th May, **5** – 06th Jun, **7** – 16th Jun, **9** – 26th Jun, **11** – 07th Jul, **14** – 17th Jul, **15** – 28th Jul, **16** – 07th Aug; **Dates M2: 2** - 19th May, **4** – 30th May, **5** – 06th Jun, **8**- 16th Jun, **10** – 26th Jun, **12** – 07th Jul, **14** – 17th Jul, **17** – 07th Aug.

Figure III.14: RDA plot of zooplankton groups and environmental variables in the 2 maize sampling sites.

In the control site (sp6 x env 3), the RDA accepted model revealed 5 environmental variables influencing the zooplankton communities in all maize sampling sites (Table III.8), metolachlor is the variable more correlated with the Axis 1. This axis explains 49,9% of total variation.

Table III.9: Correlations of the environmental variables with the canonical axes from sp6 x env3 RDA; influence as a percentage of total variation for the corresponding axis; stronger correlations in bold.

Environmental variables	Correlation with canonical axes	
	Axis 1 (49,9%)	Axis 2 (11,6 %)
metolachlor	**-0.39**	0.06
AMPA	0.35	**0.41**
glyphosate	0.35	**0.41**
Shannon	0.24	**0.75**
Margalef	**0.33**	0.06

Regarding the Control site (R) RDA triplot, the dates: 7th of Jul and 30th of May show higher rotifer count when glyphosate and AMPA values are higher two. On the 19th of May higher glyphosate values are shown at the same time than the indexes of Shannon and Margalef show high values too.

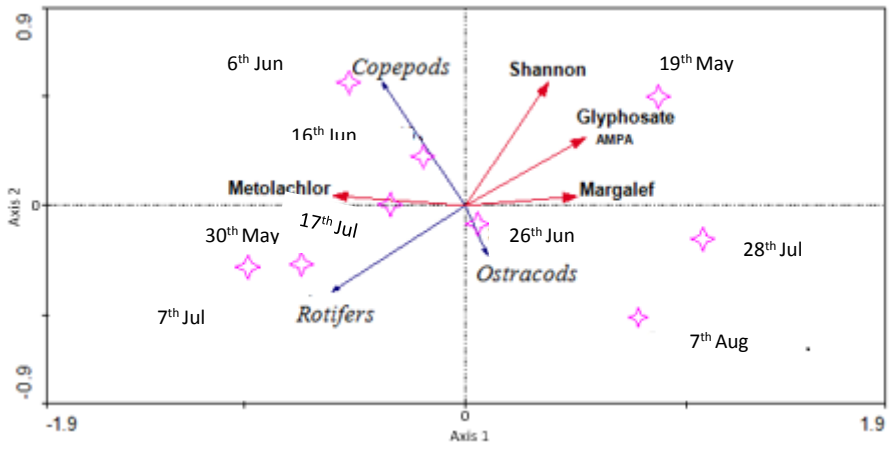


Figure III.15: RDA plot of zooplankton groups and environmental variables in the R sampling site.

4 Discussion

Zooplankton composition, abundance and diversity

A total of 37 species of rotifers were identified. From these 19, in the two maize sampling sites (M1 and M2), 25 in the three tomato sampling sites (T1, T2 and T3) and 24 in the R sampling site. Ostracods were present in all sampling sites as well as the three development stages of copepods (nauplius, copepodite and adult). Two families of Cladocera were identified (Chydoridae e Moinidae). Chydoridae was observed in two tomato sites (T1 and T3) and Moinidae in the control site (R). Since there are repeated species of rotifers between the control site (R) and both tomato and maize crops T1, T2,T3, M1 and M2, and also the presence of ostracods and copepods in all sites, it can be assumed that the control (R) site can serve as a recolonization spot for the others. According to (Meers, 2009) zooplankton is able to move twice 30-40 m every day. Nauplii and rotifer were the major component of zooplankton in all sampling sites, according to (Paggi, 1997) rotifers and nauplii are generally more tolerant to the effect of pesticides. The presence of pesticides alters the structure patterns of the zooplankton community, by reduction or elimination of macrozooplankton allowing rotifers to highly increase their densities. These results show a dominance by smaller zooplankton that according to (Hanazato, 2001) might be an inducted consequence of pesticide use. Rotifer densities were amongst all zooplankton the higher in all sampling sites, this might be explained due to, Cladocera beeing an effective suppressor of rotifer densities, unlike copepods that play a minor role in this suppression (Nogrady *et al*., 1993, Fussmann, 1996) and in all sampling sites cladoceran densities were very low. Copepod densities were the second highest after rotifer, Gliwicz (1994) reported that the presence of copepods reduces the growth rate of Cladocera. Therefore, copepods and cladocerans appear to play a role in rotifer densities. Even though not all zooplankton present in the maize and tomato sampling sites are also found in the control (R) site and adult copepods and cladoceran are present in much lower numbers, it can't be only associated to the presence of pesticides in the water of the sampling sites. According to (Meester, 2009) Diel vertical migration is a conspicuous and widespread behavior associated to zooplankton. The animals reside higher in the water column during the night than during the day. During the day they are at deeper water layers while during the night they distribute more evenly allowing to be caught in higher numbers. Different life stages may differ in their daytime distribution and migration pattern. In this study, all the samples were collected during the day so there is the hypothesis that some organisms could be present in higher numbers if the sampling occurred also during the night time.

Pesticides influence on zooplankton

The multiple regression model that was performed didn't allow to establish strong relationships between the pesticides and the variation of diversity and richness indexes. In the tomato crop, the biological variation of organisms along time didn't seem to be influenced by the pesticides present on the water. Regarding the maize crop, there were neagative effects on diversity due to pesticides as illustrated by the 3 indexes (decrease in biodiversity) namely by the presence of the insecticide chlorpyrifos (AChE inhibitor, D.magna 48h, EC50: 0.0003 (mg L-1)) but only in the sample from (19th May) that had a very high residue (12 ug/l). Studies with chlorpyrifos at 10 ug/l concentrations showed to affect drastically all macrozooplacton and that nauplii and rotifers weren't affected raising their density 5 to 20 times (Hurlbert, 1972) which corroborates the results of this study.

Regarding the Tomato RDA triplot, the dates 23 – 07th Aug of T1 sampling site and 24 – 07th Aug of the T2 sampling site show that higher values 4,5 ug/l for T1 and 3,7 for T2 of chlorantrinaprole (activator of insect ryanodine receptors; D.magna 48h, EC50: 0.0116 (mg L-1)) and (0,96 ug/l for T1) metribuzin (inhibitor of photosynthesis photosystem II; D.magna 48h, EC50: 49 (mg L-1)) are negatively correlated to the number of Copepods and the Pielou Index. The dates: 19 – 17th Jul of the T1 sampling site and 20 – 17th Jul, 25 – 07th Aug of the T3 sampling site, show that high glyphosate (Inhibitor of essential EPSPS; D.magna 48h, EC50: 40 (mg L-1)) values (2,3 ug/l for T1 and 2,6-3,9 ug/l for T3) are negatively correlated with the number of Cladocerans and Ostracods. The period of time that most herbecides stay in water show that they will cause serious adverse effects in the populations of freshwater zooplankton (Newbold, 1975).

Regarding the Maize RDA triplot, higher values of glyphosate (0,66 ug/l), Ampa (0,88 ug/l) and Phosphate (2,38 mg/l) seem to be linked to lower Margalef index values in the dates (**3** – 30th May), (**5** – 06th Jun), (**9** – 26th Jun) and (11 – 07th Jul) of the M1 sampling site, since Margalef index vector is diametrically opposed to the vectors of glyphosate , Ampa, Phosphate. Investigation results of Stemberger and Lazorchak (1994) nutrients (as indicated by total phosphorus) is a significant predictor of zooplankton assemblages. Specifically, high nutrient levels were indicative of zooplankton communities dominated by small-bodied species. Experimental pond experiments have demonstrated that herbicide exposure indirectly affects zooplankton community structure and abundance (DeNoyelles *et al*., 1982). Since it was not possible to establish significant links between the environmental parameters analyzed and the zooplankton there is an enforcement of the idea that multiple factors are responsible for the ecological responses in zooplankton communities besides pesticides. Such factors include a wide range of stressors that increase the

rate of acute and chronic effects due to toxicants (factor of increase in brackets): increased temperature (10) (Song *et al.* 1997, Osterauer and Köhler 2008), food limitation (2) (Chandini 1988, Pieters *et al.* 2005), increased salinity (10) (Wildgust and Jones 1998), low oxygen (2) (Van der Geest et al. 2002), UV radiation (30) (Liess et al. 2001), and competition (10) (Liess 2002) leading to a reduction in zooplankton fitness and growth. These factors occur naturally and balanced under natural conditions. However, most of the sensitivities of different species to toxicants have been analysed within test systems that are characterised by favourable conditions. So the resulting estimations of toxicological sensitivities, are often used as the only basis for risk assessments putting aside the interaction between environmental and toxicological variables. The sensitivity to toxicants can be changed by many abiotic and biotic factors, as was reviewed by Heugens *et al*. (2001). These effects can result in a so called ‘‘context sensitivity’’ that is very different from the toxicological sensitivity (Liess and Beketov 2011).

5 Conclusions

The Maize sampling sites (M1, M2) were more abundant in rotifers, copepods and ostracods than the Tomato sampling site (T1, T2 and T3). Pesticides seem to change the structure of the zooplankton community, by affecting in more extent the copepod adult stages and the cladocerans allowing rotifers and nauplii to achieve higher densities. The insecticide chlorpyrifos at the concentration 12 ug/l was observed to generate a high increase in the rotifer density by negatively affecting the macro zooplankton densities. Values of 3.5-4.7 ug/l of chlorantrinaprole and 0.96 ug/l of metribuzin seem to negatively affect the size of the copepod community. Cladoceran and Ostracod comunnities seem to decrease when glyphosate values are in the range of 2,3-3,9 ug/l. Values of glyphosate (0,66 ug/l), Ampa (0,88 ug/l) and Phosphate (2,38 mg/l) seem to be linked to lower Margalef index values (species richness). The data obtained in this field study represents an important contribution to the knowledge of Ecological risk assessment of pesticides for the aquatic medium. By the enrichment and validation of prior knowledge.

CHAPTER IV

FINAL REMARKS

1. Challenges regarding future studies

In order to improve the knowledge of the possible impact of pesticides in the main national agriculture areas of the country, it's important to develop new studies under natural conditions that allow the better understanding of the ecology of zooplankton communities. Before understanding the effects of pesticides, the ecological behavior of zooplankton under different natural influences should be better studied. By doing so, maybe better understanding of pesticide effects could be achieved. The following topics are given as suggestions for future researches:

- Knowledge of the daily migrant movements in the water column;
- Study and compare the ecology of zooplankton in areas under different environmental influences and food availability and after, test the toxicity of pesticides in such conditions;
- Study the composition of the zooplankton community in shade and light conditions and water column depth.
- Better understand the role of resting eggs in recolonization and recovery.

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Annexes

Annex I - Fungicides registered in Portugal for tomato 2014

Diseases	Fungicides (active ingredient)	
Alternariosis	azoxistrobine	famaxadon + macozeb
	azoxistrobine + difenoconazol	folpet
	captan	mancozeb;
	clortalonil	dimethomorph + piraclostrobin
	cimoxanil + clortalonil	cimoxanil + fosetil + copper
	(oxicloride)	
	difenoconazol	
Antracnosis	folpet;	
Bacteriosis	copper (hydroxide)	copper (oxicloride)
Pseudomonas spp.		
Cladosporiosis	azoxistrobine + difenoconazol	captan
	folpet	mancozeb
	propineb	
Mildew	ametoctradin + dimethomorph	azoxistrobin
	benalaxil + macozeb	benalaxil-M + mancozeb
	bentiavalicarb-isopropil + mancozeb	captan
	ciazofamid	cimoxanil + clortalonil
	cimoxanil + copper (ocicloride)	cimoxanil + copper (copper sulfate and calcium)
	cimoxanil + famoxadon	
	cimoxanil + folpet + mancozeb	cimoxanil + folpet
	cimoxanil + fosetil + copper (oxicloride)	cimoxanil + clortalonil
	cimoxanil + mancozeb	

Annex I - Fungicides registered in Portugal for tomato 2014 (Cont.)

Diseases	Fungicides (active ingredient)	
Mildew	cimoxanil + copper oxicloride	cimoxanil + copper ocicloride + propineb
	cimoxanil + propineb	clotalonil
	copper (hidroxide)	copper (hydroxide) + melataxil
	copper (oxicloride) + iprovalicarb	copper (tribasic copper sulfate)
	copper (copper and calcium sulfate)	dimethomorph
	dimethomorph + mancozeb	dimethomorph + piraclostrobin
	dimetomorph + folpet	folpet + iprovalicarb
	famozadon + mancozeb	mancozeb + metalaxil
	dimethomorph + metiram	mandipropamid
	folpet	propineb
	mancozeb	
	mancozeb + metalaxil-m	
	metiram	
		azoxistrobin + defenconazol
Plant wilting	propamocarb (hidrocloride)	ciflufenamid
		penconazol
Powdery mildew	azoxistrobin	
	azoxistrobin + tebuconazol	
	sulphur	
	potassium hydrogen carbonate	
Grey mold	ciprodinil + fludioxonil	fenehexamid
	hyprodion	penthiopyrad
	pyrimethanil	
Septoria	captan	mancozeb

Annex II - Insecticides registered in Portugal for tomato 2014

Pests	Insecticides (active ingredient)	
"Alfinetes"	lambda-cyhalothrin	thiamethoxam
Mites	abamectin + clorantraniliprole hexitiaxox	sulphur
"Ácaros tetraniquídeos"	abamectin clofentezine	acrinathrin etoxazole
Aphids	acetamiprid cypermethrin deltametrin esfenvalerate imidacloprid imidacloprid + lambda - cyalothrin pymetozine	alpha-cypermethrin cyfluthrin + imidacloprid dimethoate spirotetramat lambda – cyalothrin thiamethoxam
Caterpillars	abamectin + clorantraniliprole beta – cyfluthrin cyfluthrin + imidacloprid clorantraniliprole chlorpyrifos deltametrin indoxacarb imidacloprid + lambda – cyalothrin lufenuron spinosad emamectin	alpha – cypermethrin cyfluthrin cypermethrin methyl – chlorpyrifos + deltametrin lambda – cyalothrin lambda – cyalothrin + thiamethoxam methomyl

Annex II - Insecticides registered in Portugal for tomato 2014 (Cont.)

Pests	Insecticides (active ingredient)	
"Larvas mineiras"	abamectin	abamectin + clorantraniliprole
	azadirachtin	cyromazine
	methomyl	thiamethoxam
Whiteflies	acetamiprid	alfha – cypermethrin
	azadirachtin	cypermethrin
	deltamethrin	spirotetramat
	imidacloprid	lambda – cyalothrin + thiamethoxame
	methomyl	thiametoxam
	pymetrozine	azadirachtin
		cyfluthrin
"Nóctuas"	alpha- cypermethrin	indoxacarb
	deltamethrin	
	lambda – cyalothrin	
"Percevejo"	deltamethrin	
Scutigerela	chlorpyrifos	
		clorantraniliprole
Tomato leafminer	abamectin + clorantraniliprole	indoxacarb
	emamectine	spinosad
	methomyl	
		acrinathrin
Thrips	abamectin	lufenuron
	deltamethrin	
		deltamethrin
Californian thrips	acrinathrin	methiocarb
Western flower thrips	lufenuron	

Annex III - Herbicides registered in Portugal for tomato 2014

Weeds (Class)	Herbicides (active ingredient)	
Monocots	fluazifop-p-butyl	quizalofop-p-ethyl
	s-metolachlor	
Monocots/Dicots	cycloxydim	diquat
	flufenacet + metribuzin	glyphosate (ammonium salt)
	glufosinate ammonium	metribuzin
	pendimethalin	rimsulfuron

Annex IV - Fungicides and Insecticides registered in Portugal for maize 2014

Diseases	Fungicides (active ingredient)	
"Ferrugem"	epoxiconazole + pyraclostrobin	
Helmintosporiosis	epoxiconazole + pyraclostrobin	
Pests		
Aphids	deltametrhin	
"Alfinete"	chlorpyrifos	lambda cyhalothrin
	tefluthrin	
"Brocas"	cypermethrin	deltamethrin
	indoxacabr	
"Nóctuas"	alpha-cypermethrin	beta cyfluthrin
	deltamethrin	lambda - cyhalothrin
	tefluthrin	
"Percevejo"	deltamethrin	
"Piral"	alpha-cypermethrin	cypermethrin
	deltamethrin	lambda-cyhalothrin
Scutigerela	chlorpyrifos	tefluthrin
Sesamia	cypermethrin	deltamethrin
Triphs	deltamethrin	

Annex V - Herbicides registered in Portugal for maize in 2014

Weeds (Class)	Herbicides (active ingredient)	
Grass / Dicots	dicamba + nicosulfuron + rinsulfuron	dimethenamid
	dimethenamid – p + terbuthylazine	mesotrione + terbuthylazine
	nicosulfuron	nicosulfuron + rinsulfuron
	nicosulfuron + terbuthylazine	pendimethalin
	s-metolachlor	tembotrione - isoxadifen-ethyl
Monocots/Dicots	2,4-D + glyphosate	bentazone
	bentazone + terbuthylazine	diquat
	flufenacet + terbuthylazine	foramsulfuron + isoxadifen-ethyl
	foramsulfuron-sodium + thiencarbazone methyl + cyprosulfamide	glyphosate (isopropylammonium salt)
	linuran	mesotrione
	mesotrione + s-metolachlor + benoxacor	nicosulfuron
	pyridate	pendimethalin
	sulcotrione	S-metolachlor + terbuthylazine
		2,4-D + bromoxinil (octanoate)
Dicots	2, 4-D ethylhexyl ester + florasulam	bentazone + dicam
	bentazone	bromoxinil (octanoate)
	bromoxinil	bromoxonilil + prosulfuron
	bromoxinil (butyric ester)	clopyralid
	bromoxinil + terbuthylazine	dicam + tritosulfuron
	dicam (diethylammonium salt)	fluroxypyr
	dicam + prosulfuron	
	prosulfuron	

Annex VI - Pesticide residue values on the irrigation water of M1 sampling site

Pesticides M1	19/05/14	20/05/14	06/06/14	16/06/14	26/06/14	07/07/14	17/07/14	28/07/14	07/08/14
Insecticides									
cypermethrin (Sodium channel modulators)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	*10*	< 0.1
Herbicides/metabolites									
glyphosate (Inhibition of essential EPSPS)	< 0.05	< 0.05	< 0.05	*0.77*	< 0.05	< 0.05	< 0.05	*0.43*	*0.66*
AMPA (Unknown MoA)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	*1.2*	*0.88*
metolachlor (Inhibition of mitosis and cell division)	< 0.05	*0.57*	*0.19*	*2.8*	*0.11*	< 0.05	< 0.05	< 0.05	< 0.05
terbuthylazine (Inhibits photosynthesis (photosystem II))	1.9	*1.2*	*1.2*	*1.8*	*0.58*	*0.24*	*0.093*	*0.28*	*0.067*
desethyl-terbuthylazine (Unknown MoA)	0.6	*0.47*	*0.25*	*0.91*	*0.78*	*0.15*	*0.29*	*0.65*	< 0.05
Sum	2.5	*2.24*	*1.64*	*6.28*	*1.47*	*0.39*	*0.383*	*12.56*	*1.607*

Annex VII - Pesticide concentration on the irrigation water of M2

Pesticides	*19/05/14*	*30/05/14*	*06/06/14*	*16/06/14*	*26/06/14*	*07/07/14*	*17/07/14*	*28/07/14*	*07/08/14*
Insecticides									
chlorpyrifos (-ethyl) [AChE inhibitor)	*12*	*0.16*	*0.16*	*0.73*	< 0.1	< 0.1	< 0.1	< 0.1	*2*
Herbicides/metabolites									
glyphosate (Inhibition of essential EPSPS)	*1*	< 0.05	< 0.05	*0.77*	*0.19*	*0.17*	*1.1*	*0.33*	*0.66*
AMPA (Unknown MoA)	*0.92*	< 0.05	< 0.05	*1*	*0.49*	*0.38*	*1.2*	*1.1*	*0.79*
metolachlor (Inhibition of mitosis and cell division)	1.4	0.27	0.11	2.8	0.1	< 0.05	< 0.05	< 0.05	0.34
terbuthylazine (Inhibits photosynthesis (photosystem II))	8.5	2.8	2.8	1.7	0.54	0.31	< 0.05	< 0.05	< 0.05
desethyl-terbuthylazine (Unknown MoA)	1.1	0.4	0.4	0.89	0.3	0.5	< 0.05	< 0.05	< 0.05
Sum	24.92	3.63	3.47	7.89	1.62	1.36	2.3	1.43	3.79

Annex VIII - Pesticide concentration on the irrigation water of T1

Pesticides	19/05/14	20/05/14	06/06/14	16/06/14	26/06/14	07/07/14	17/07/14	28/07/14	07/08/14
Insecticides									
imidacloprid (Blockage of the nicotinergic neuronal pathway)	-	-	-	< 0.05	< 0.05	*0.098*	*0.14*	< 0.05	< 0.05
indoxacarb (Neuronal sodium channels blocker)	-	-	*3*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
chlorantraniliprole (Activator of insect ryanodine receptors)	-	-	-	< 0.05	< 0.05	*4.5*	*1.9*	*0.65*	*0.65*
Herbicides/metabolites									
glyphosate (Inhibition of essential EPSPS)	*5*	*1*	*0.5*	*2*	*2.3*	*2.2*	*2.3*	*3.3*	*3.9*
AMPA (Unknown MoA)	*16*	*9*	*5*	*8.9*	*7.5*	*7*	*4*	*3*	*2.1*
metribuzin [Inhibits photosynthesis (photosystem II)]	*0.13*	*0.17*	< 0.05	< 0.05	< 0.05	*0.96*	< 0.05	< 0.05	< 0.05
Sum	*21.3*	*10.17*	*8.5*	*10.9*	*9.8*	*14.758*	*8.34*	*6.95*	*6.65*

Annex IX: Pesticide concentration on the irrigation water of T2

Pesticides	*19/05/14*	*20/05/14*	*06/06/14*	*16/06/14*	*26/06/14*	*07/07/14*	*07/08/14*
Insecticides							
imidacloprid (Blockage of the nicotinergic neuronal pathway)	-	-	-	< 0.05	2.4	0.44	< 0.05
indoxacarb (Neuronal sodium channels blocker)	-	-	1.7	< 0.1	< 0.1	< 0.1	< 0.1
chlorantraniliprole (Activator of insect ryanodine receptors)	-	-	-	0.15	0.29	3.7	0.65
Fungicides							
cymoxamil (Unknown MoA)	0.061	0.061	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Herbicides/metabolites							
glyphosate (Inhibition of essential EPSPS)	5.4	1.2	1	2.6	2.8	2.8	-
AMPA (Unknown MoA)	16	9.4	5.1	9.9	7.7	7	-
metribuzin [Inhibits photosynthesis (photosystem II)]	0.12	0.061	< 0.05	< 0.05	< 0.05	< 0.05	0.33
Sum	21.58	10.72	7.8	12.5	13.19	13.94	0.98

Annex X: Pesticide concentration on the irrigation water of T3

Pesticides	*19/05/14*	*20/05/14*	*06/06/14*	*16/06/14*	*26/06/14*	*07/07/14*	*17/07/14*	*28/07/14*	*07/08/14*
Insecticides									
imidacloprid (Blockage of the nicotinergic neuronal pathway)	-	-	-	< 0.05	< 0.05	< 0.05	*0.11*	*2.6*	*0.17*
indoxacarb (Neuronal sodium channels blocker)	-	-	*1.4*	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*	*< 0.1*
chlorantraniliprole (Activator of insect ryanodine receptors)	-	-	-	< 0.05	< 0.05	< 0.05	*1.7*	*1.7*	*2*
Herbicides/metabolites									
glyphosate (Inhibition of essential EPSPS)	*5.1*	*1.1*	*0.6*	*2.1*	*2.6*	*2.6*	*2.6*	*3.3*	*3.9*
AMPA (Unknown MoA)	*15*	*9.3*	*4.8*	*8.7*	*7.7*	*7*	*4*	*3*	*2.1*
metribuzin [Inhibits photosynthesis (photosystem II)]	*0.13*	*0.13*	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Sum	*20.23*	*10.53*	*6.8*	*10.8*	*10.3*	*9.6*	*8.41*	*10.6*	*8.17*

Annex XI – Selected Insecticides active ingredient characteristics: physico-chemical properties and environmental characteristics

Insecticides (a.i)	Sw 20ºC (mg L-1)	VP (mPa)	*H (Pa m3 mol-1)*	*Kow*	*Koc (mL g-1)*	*DT50lab soil (d) (20ºC)*	*DT50fiel d soil (d)*	*Aqueous photolysis DT50 (days) at pH 7*	*Aqueous hydrolysis DT50 (days) at 20oC and pH 7*	*Water-sediment DT50 (days)*	*Water phase only DT50 (days)*
cypermethrin	0.009	2.3E-04	2.00E-02	5.3	156250	68	69	13	179	17	3
chlorpyrifos (ethyl)	1.05	1.43	0.478	4.7	8151	76	21	29.6	25.5	36.5	5
imidacloprid	610	4.0E-07	1.7E-10	0.57	-	187	174	0,2	Stable	129	30
indoxacarb	0.2	0.006	6.00E-05	4.65	6450	5	20	3	22	6	1.4
chlorantraniliprole	0.88	6.3 X 10-09	3.2 X 10-09	2.86	362	597	204	0.31	Stable	170	23.5

Annex XII – Selected Fungicides active ingredient characteristics: physico-chemical properties and environmental characteristics

Fungicides (a.i)	Sw 20ºC (mg L-1)	VP (mPa)	*H (Pa m3 mol-1)*	*Kow*	*Koc (mL g-1)*	*DT50lab soil (d) (20ºC)*	*DT50fiel d soil (d)*	*Aqueous photolysis DT50 (days) at pH 7*	*Aqueous hydrolysis DT50 (days) at 20oC and pH 7*	*Water-sediment DT50 (days)*	*Water phase only DT50 (days)*
cymoxanil	780	0.15	3.80 X 10-05	0.67	-	1.4	3.5	1.7	1.1	0.3	0.3

Annex XIII - Selected Herbicides active ingredient characteristics: physico-chemical properties and environmental characteristics

Herbicides (a.i)	Sw 20ºC (mg L-1)	VP (mPa)	*H (Pa m3 mol-1)*	*Kow*	*Koc (mL g-1)*	*DT50lab soil (d) (20ºC)*	*DT50fiel d soil (d)*	*Aqueous photolysis DT50 (days) at pH 7*	*Aqueous hydrolysis DT50 (days) at 20oC and pH 7*	*Water-sediment DT50 (days)*	*Water phase only DT50 (days)*
glyphosate	10500	0.0131	2.10 X 10-07	-3.2	1424	15.3	23.79	69	Stable	74.5	9.9
metolachlor	530	1.7	2.40 X 10-03	3.4	120	15	21	Stable	Stable	365	88
terbuthylazine	6.6	0.12	3.24 X 10-03	3.4	-	75.1	22.4	Stable	Stable	70	6
metribuzin	1165	0.121	2.00 X 10-05	1.65	-	11.5	19	0.2	Stable	50	41

Annex XIV - Selected Insecticides active ingredient ecotoxicological data

Insecticides (a.i)	*Cladocera*		*Algae Species*	*72h EC50 (mg L-1)*	*Other crustaceans*
	D. magna 48h EC_{50} (mg L^{-1})	*D. magna 21 d NOEC (mg L^{-1})*			*Americamysis bahia* LC50 (mg l^{-1})
cypermethrin	0.0003	0.00004	Pseudokirchneriella subcapitata	> 0.1	0.0128
chlorpyrifos (ethyl)	0.0001	0.0046	Unknown species	-	0.00004
imidacloprid	85	1.8	*Scenedemus subspicatus*	> 10	0.034
indoxacarb	0.6	0.042	*Raphidocelis subcapitata*	0.11	-
chlorantraniliprole	0.0116	0.00447	*Pseudokirchneriella subcapitata*	> 4.0	-

Annex XV - Selected Fungicides active ingredient aquatic ecotoxicological data

Fungicides (a.i)	*Cladocera*		*Algae Species*	*72h EC50 (mg L-1)*	*Other crustaceans*
	D. magna 48h EC_{50} (mg L^{-1})	*D. magna 21 d NOEC (mg L^{-1})*			*Americamysis bahia* LC50 (mg l^{-1})
cymoxanil	27	0.067	Anabaena flos-aquae	0.254	44.4

Annex XVI - Selected Herbicides active ingredient aquatic ecotoxicological data

Herbicides (a.i)	*Cladocera*		*Algae Species*	*72h EC50 (mg L-1)*	*Other crustaceans*
	D. magna 48h EC_{50} (mg L^{-1})	*D. magna 21 d NOEC (mg L^{-1})*			*Americamysis bahia* LC50 (mg l^{-1})
glyphosate	40	30	*Scenedesmus quadricauda*	4.4	40
metolachlor	23.5	0.707	*Pseudokirchneriella subcapitata*	57.1	4.2
terbuthylazine	21.2	0.019	*Pseudokirchneriella subcapitata*	0.012	0.167
metribuzin	49	0.32	*Scenedemus subspicatus*	0.02	-

Annex XVII - Zooplankton abundance regarding the tomato crop in the three sampling sites along time

	GROUP ABUNDANCE				TOTAL ABUNDANCE
SAMPLING DATE	**Rotifer**	**Copepod**	**Ostracod**	**Cladocera**	
T1 19.05.14	231	62	35	9	337
T1 30.05.14	883	195	100	0	1178
T1 06.06.14	1161	305	501	0	1967
T1 16.06.14	2218	105	65	0	2388
T1 26.06.14	2165	15	155	0	2335
T1 07.01.14	342	0	15	0	357
T1 17.07.14	225	13	0	0	238
T1 28.07.14	1003	5	5	0	1013
T1 07.08.14	476	0	12	0	488
T2 19.05.14	115	77	5	0	197
T2 30.05.14	214	58	19	0	291
T2 06.06.14	128	39	16	0	183
T2 16.06.14	144	31	30	0	205
T2 26.06.14	298	24	198	0	520
T2 07.07.14	233	58	86	0	377
T2 07.08.14	23	1	14	0	38
T3 19.05.14	41	337	0	0	378
T3 30.05.14	185	142	15	0	342
T3 06.06.14	110	118	29	0	257
T3 16.06.14	490	29	42	3	564
T3 26.06.14	430	58	9	0	497
T3 07.07.14	1369	119	16	0	1504
T3 17.07.14	123	38	0	0	161
T3 28.07.14	2397	332	7	0	2736
T3 07.08.14	149	30	4	0	183
T1+T2+T3	15153	2191	1378	12	18734

Annex XVIII - Zooplankton abundance regarding the maize crop in the two sampling sites along time

	Group Abundance			*Total Abundance*
Sampling date	**Rotifer**	**Copepod**	**Ostracod**	
M1 19.05.14	627	535	0	1162
M1 30.05.14	509	1616	13	2138
M1 06.06.14	2030	2377	22	4429
M1 16.06.14	2005	301	89	2395
M1 26.06.14	472	1545	53	2070
M1 07.07.14	179	4518	44	4741
M1 17.07.14	223	2402	0	2625
M1 28.07.14	608	914	0	1522
M1 07.08.14	326	156	27	509
M2 19.05.14	2659	24	20	2703
M230.05.14	1008	151	0	1159
M2 06.06.14	2765	327	0	3092
M2 16.06.14	12364	62	0	12426
M2 26.06.14	15654	91	0	15745
M2 07.07.14	18684	128	0	18812
M2 17.07.14	1323	0	25	1348
M2 07.08.14	754	0	0	754
M1 + M2	62190	15147	293	77630

Annex XIX - Zooplankton abundance regarding R sampling site in the along time

	Group Abundance				*Total Abundance*
Sampling date	**Rotifer**	**Copepod**	**Ostracod**	**Cladocera**	
19.05.14	43	15	0	0	58
30.05.14	1796	25	0	17	1838
06.06.14	604	113	0	35	752
16.06.14	790	133	0	5	928
26.06.14	434	23	0	10	467
07.07.14	6060	94	0	0	6154
17.07.14	111	2	7	0	120
28.07.14	67	5	2	0	74
07.08.14	184	3	2	0	189

ANNEX XX – Aplied Pesticides in "Caldas 5 / 2014 / Tomate de Indústria"

Data da aplicação	Justificação da Aplicação (Doença, Praga, Infestantes)	Produto aplicado					Medidas a tomar e justificação			IS	Operador	Assinatura
		Nome do produto	APV nº	substância ativa	Fornecedor	Lote	Dose / Ha	Volume Calda (L/Ha)	Pulverizador			
15-05-2014	Infestantes	TITUS	2702	rimsulfurão			0,04	400,00	Pulverizador 1	45	João Geada	BM
15-05-2014	Monocotiledóneas e dicotiledóneas	ECLIPSE WG	3866	metribuzina			0,45	400,00	Pulverizador 1	60	João Geada	BM
22-05-2014	Míldio	MELODY	3801	iprovalicarbe folpete			1,30	400,00	Pulverizador 1	23	João Geada	BM
22-05-2014	Mosca branca	KARATE ZEON	0020	lambda-cialotrina			0,20	400,00	Pulverizador 1	6	João Geada	BM
28-05-2014	Infestantes	TITUS	2702	rimsulfurão			0,04	400,00	Pulverizador 1	45	João Geada	BM
28-05-2014	Monocotiledóneas e dicotiledóneas	ECLIPSE WG	3866	metribuzina			0,50	400,00	Pulverizador 1	60	João Geada	BM
31-05-2014	Lagartas	STEWARD	0093	indoxacarbe			0,13	400,00	Pulverizador 1	6	João Geada	BM
31-05-2014	Míldio	DUETT-M	3470	manoozebe cimoxanil			3,00	400,00	Pulverizador 1	50	João Geada	BM
09-06-2014	Lagartas	KARATE ZEON	0020	lambda-cialotrina			0,20	400,00	Pulverizador 1	6	João Geada	BM
09-06-2014	Míldio	VITIPEC	3373	folpete cimoxanil			1,50	400,00	Pulverizador 1	10	João Geada	BM
19-06-2014	Míldio	BRAVO 500	3460	clortalonil			3,00	400,00	Pulverizador 1	7	João Geada	BM
19-06-2014	Oídio	KUMULUS S	1259	enxofre			3,00	400,00	Pulverizador 1	Não tem	João Geada	BM
19-06-2014	Traça	AFFIRM	4029	emamectina (benzoato)			1,50	400,00	Pulverizador 1	6	João Geada	BM
28-06-2014	Míldio	MELODY	3801	iprovalicarbe folpete			1,30	400,00	Pulverizador 1	23	João Geada	BM
28-06-2014	Traça	AFFIRM	4029	emamectina (benzoato)			1,50	400,00	Pulverizador 1	6	João Geada	BM
07-07-2014	Lagartas	CORAGEN	4020	clorantraniliprol			0,20	400,00	Pulverizador 1	6	João Geada	BM
07-07-2014	Míldio	VITIPEC	3373	folpete cimoxanil			1,50	400,00	Pulverizador 1	10	João Geada	BM
24-07-2014	Lagartas	CORAGEN	4020	clorantraniliprol			0,20	400,00	Pulverizador 1	6	João Geada	BM
24-07-2014	Míldio	CABRIO DUO	0196	piraclostrobina dimetomorfe			2,50	400,00	Pulverizador 1	13	João Geada	BM
24-07-2014	Oídio	KUMULUS S	1259	enxofre			3,00	400,00	Pulverizador 1	Não tem	João Geada	BM

ANNEX XXI – Aplied Pesticides in “Caldas 8 / 2014 / Tomate de Indústria”

Data da aplicação	Justificação da Aplicação (Doença, Praga, Infestantes)	Produto aplicado					Medidas a tomar e justificação			IS	Operador	Assinatura
		Nome do produto	APV nº	substância ativa	Fornecedor	Lote	Dose / Ha	Volume Calda (L/Ha)	Pulverizador			
15-05-2014	Infestantes	TITUS	2702	rimsulfurão			0,04	400,00	Pulverizador 1	45	João Geada	BM
15-05-2014	Monocotiledóneas e dicotiledóneas	ECLIPSE WG	3866	metribuzina			0,45	400,00	Pulverizador 1	60	João Geada	BM
22-05-2014	Míldio	MELODY	3801	iprovalicarbe folpete			1,30	400,00	Pulverizador 1	23	João Geada	BM
22-05-2014	Mosca branca	KARATE ZEON	0020	lambda-cialotrina			0,20	400,00	Pulverizador 1	6	João Geada	BM
28-05-2014	Infestantes	TITUS	2702	rimsulfurão			0,04	400,00	Pulverizador 1	45	João Geada	BM
28-05-2014	Monocotiledóneas e dicotiledóneas	ECLIPSE WG	3866	metribuzina			0,50	400,00	Pulverizador 1	60	João Geada	BM
31-05-2014	Lagartas	STEWARD	0093	indoxacarbe			0,13	400,00	Pulverizador 1	6	João Geada	BM
31-05-2014	Míldio	DUETT-M	3470	mancozebe cimoxanil			3,00	400,00	Pulverizador 1	50	João Geada	BM
09-06-2014	Lagartas	KARATE ZEON	0020	lambda-cialotrina			0,20	400,00	Pulverizador 1	6	João Geada	BM
09-06-2014	Míldio	VITIPEC	3373	folpete cimoxanil			1,50	400,00	Pulverizador 1	10	João Geada	BM
19-06-2014	Míldio	BRAVO 500	3460	clortalonil			3,00	400,00	Pulverizador 1	7	João Geada	BM
19-06-2014	Oídio	KUMULUS S	1259	enxofre			3,00	400,00	Pulverizador 1	Não tem	João Geada	BM
19-06-2014	Traça	AFFIRM	4029	emamectina (benzoato)			1,50	400,00	Pulverizador 1	6	João Geada	BM
28-06-2014	Míldio	MELODY	3801	iprovalicarbe folpete			1,30	400,00	Pulverizador 1	23	João Geada	BM
28-06-2014	Traça	AFFIRM	4029	emamectina (benzoato)			1,50	400,00	Pulverizador 1	6	João Geada	BM
07-07-2014	Lagartas	CORAGEN	4020	clorantraniliprol			0,20	400,00	Pulverizador 1	6	João Geada	BM
07-07-2014	Míldio	VITIPEC	3373	folpete cimoxanil			1,50	400,00	Pulverizador 1	10	João Geada	BM
24-07-2014	Lagartas	CORAGEN	4020	clorantraniliprol			0,20	400,00	Pulverizador 1	6	João Geada	BM
24-07-2014	Míldio	CABRIO DUO	0196	piraclostrobina dimetomorfe			2,50	400,00	Pulverizador 1	13	João Geada	BM
24-07-2014	Oídio	KUMULUS S	1259	enxofre			3,00	400,00	Pulverizador 1	Não tem	João Geada	BM

ANNEX XXII - Rotifer count along time in T1 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
***Brachionus calyciflorus* (Pallas, 1766)**	0	8	0	0	0	0	0	0	0	8
Brachionus rotundiformis (Tschugunoff, 1921)	0	36	37	0	9	33	0	0	0	115
Brachionus angularis (Gosse, 1851)	0	51	7	0	20	0	0	0	0	78
Platyias quadricornis (Ehrenberg, 1832)	13	42	26	0	0	0	0	0	0	81
Keratella tropica sp.	0	0	13	51	0	0	0	0	0	64
L. quadridentata (Ehrenberg, 1830)	0	0	0	12	152	38	22	98	13	335
Lecane sp1	0	0	69	0	0	0	4	43	0	116
Lecane sp2	0	59	27	224	476	11	0	809	271	1877
Lecane sp3	0	0	0	0	0	0	2	0	48	50
Lecane sp4	15	0	0	0	0	7	21	7	0	50
L. patella (O.F. Müller, 1786)	35	42	67	138	99	67	14	6	101	569
M. ventralis sp.	51	40	171	918	1145	153	147	40	43	2708
Filinia cornuta sp.	0	0	0	0	0	0	7	0	0	7
Rsp1	35	246	168	547	129	29	3	0	0	1157
Rsp3	0	0	0	0	14	0	5	0	0	19
Rsp4	59	336	552	317	121	0	0	0	0	1385
Rsp6	23	23	24	11	0	4	0	0	0	85

ANNEX XXIII - Copepod count along time in T1 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Nauplius	33	104	147	76	7	0	10	0	0	377
Copepodite	10	37	106	25	5	0	3	0	0	186
Adult	19	54	52	4	3	0	0	5	0	137

ANNEX XXIV - Ostracod count along time in T1 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	07/8	Sum
Ostracod	35	100	501	65	155	15	0	5	12	888

ANNEX XXV - Cladocera count along time in T1 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Family Chydoridae	9	0	0	0	0	0	0	0	0	9

ANNEX XXVI - Rotifer count along time in T2 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	7/8	Sum
Brachionus quadridentatus (Hermann, 1783)	0	0	2	1	0	0	0	3
Brachionus calyciflorus (Pallas, 1766)	0	0	0	2	5	10	0	17
Brachionus rotundiformis (Tschugunoff, 1921)	0	0	3	1	0	0	0	4
Brachionus angularis (Gosse, 1851)	0	5	0	0	0	3	0	8
Trichocerca sp.	32	124	37	19	9	8	0	229
L. quadridentata (Ehrenberg, 1830)	0	0	2	5	11	0	0	18
Lecane sp2	0	10	0	0	0	2	0	12
Lecane sp3	6	18	7	3	2	1	4	41
Lecane sp4	11	7	3	0	0	0	0	21
Polyartha sp.	2	0	0	0	0	8	0	10
L. patella (O.F. Müller, 1786)	0	0	5	37	156	28	12	238
Colurella sp.	3	0	6	7	5	0	0	21
M. ventralis sp.	0	9	2	5	14	0	2	32
Filinia cornuta	2	0	0	0	0	0	0	2
Filinia brachiata (Rousselet, 1901)	0	0	0	19	46	161	2	228
Rsp1	0	18	25	24	33	8	2	110
Rsp2	56	10	7	9	8	0	0	90
Rsp4	0	0	4	1	0	4	0	9
Rsp5	0	7	12	7	8	0	0	34
Rsp6	3	6	13	4	1	0	1	28

ANNEX XXVII - Copepod count along time in T2 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	7/8	Sum
Nauplius	77	58	36	30	24	57	0	282
Copepodite	0	0	3	1	0	1	1	6
Adult	0	0	0	0	0	0	0	0

ANNEX XXVIII - Ostracod count along time in T2 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	7/8	Sum
Ostracod	5	19	16	30	198	86	14	368

ANNEX XXIX - Rotifer count along time in T3 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Brachionus quadridentatus (Hermann, 1783)	6	0	7	74	0	0	0	0	0	87
Brachionus calyciflorus (Pallas, 1766)	0	0	4	0	0	2	0	0	0	6
Brachionus urceolaris (O.F. Müller, 1773)	0	0	14	16	0	0	0	0	0	30
Brachionus rotundiformis (Tschugunoff, 1921)	0	0	0	7	0	3	0	0	0	10
Platyias quadricornis (Ehrenberg, 1832)	0	0	0	3	0	0	0	0	0	3
Trichocerca sp.	13	112	28	17	0	0	0	0	0	170
L. quadridentata (Ehrenberg, 1830)	0	0	0	18	7	11	0	15	11	62
Lecane sp1	2	0	0	0	0	14	0	13	3	32
Lecane sp2	0	0	0	41	3	29	0	143	7	223
Lecane sp3	12	33	6	0	0	0	0	2	0	53
Polyartha sp.	1	0	0	0	0	0	0	0	0	1
L. patella (O.F. Müller, 1786)	2	0	0	82	315	1231	104	2178	96	4008
Colurella sp.	0	2	2	33	0	0	0	0	0	37
M. ventralis sp.	0	0	0	78	39	20	13	24	18	192
Filinia brachiata (Rousselet, 1901)	0	0	0	0	0	25	0	0	0	25
Rsp1	3	11	26	74	41	22	4	22	14	217
Rsp2	0	12	0	0	2	0	0	0	0	14
Rsp4	1	11	7	34	9	6	2	0	0	70
Rsp5	0	2	9	0	0	0	0	0	0	11
Rsp6	1	2	7	13	14	6	0	0	0	43

ANNEX XXX - Copepod count along time in T3 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Nauplius	337	142	117	11	55	97	38	229	24	1050
Copepodite	0	0	1	11	0	7	0	72	4	95
Adult	0	0	0	7	3	15	0	31	2	58

ANNEX XXXI - Ostracod count along time in T3 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Ostracod	0	15	29	42	9	16	0	7	4	122

ANNEX XXXII - Cladocera count along time in T3 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Family Chydoridae	0	0	0	3	0	0	0	0	0	3

ANNEX XXXIII - Rotifer count along time in M1 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Brachionus rotundiformis (Tschugunoff, 1921)	123	400	903	775	235	59	26	0	0	2521
Brachionus angularis (Gosse, 1851)	0	0	0	0	0	0	0	157	18	175
Brachionus quadridentatus (Hermann, 1783)	0	0	88	162	71	0	0	0	0	321
Brachionus urceolaris (O.F. Müller, 1773)	0	0	0	0	0	0	0	28	0	28
Ascomorpha sp.	46	20	26	0	0	0	0	33	17	142
Trichocerca sp.	34	0	283	0	74	73	36	41	74	615
Cephalodella gibba (Ehrenberg, 1830)	17	0	0	0	0	0	0	0	0	17
Synchaeta sp.	0	25	0	0	0	0	0	0	0	25
Colurella sp.	0	12	53	0	0	0	0	0	0	65
L. patella (O.F. Müller, 1786)	0	0	0	0	0	21	27	30	0	78
Filinia brachiata (Rousselet, 1901)	0	0	0	11	0	0	0	0	34	45
M. mucronata var macracantha (Gosse, 1886)	0	0	33	0	0	0	0	0	0	33
Lecane sp2	0	0	24	0	17	0	0	0	0	41
RSp1	55	0	0	153	75	26	12	15	15	351
RSp8	316	31	113	0	0	0	102	252	43	857
Rsp7	36	21	0	0	0	0	20	52	21	150
Rsp5	0	0	0	0	0	0	0	0	104	104
Rsp3	0	0	55	904	0	0	0	0	0	959
Rsp2	0	0	452	0	0	0	0	0	0	452

ANNEX XXXIV - Copepod count along time in M1 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Nauplius Stage	320	1616	2370	290	1533	4493	2393	914	156	14085
Copepodite Stage	186	0	7	11	12	25	9	0	0	250
Adult	29	0	0	0	0	0	0	0	0	29

ANNEX XXXV - Ostracod count along time in M1 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Ostracod	0	13	22	89	53	44	0	0	27	248

ANNEX XXXVI - Rotifer count along time in M2 sampling site

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	7/8	Sum
Brachionus rotundiformis (Tschugunoff, 1921)	2616	758	2284	12143	15554	18684	1233	666	53938
Tricocherca sp.	10	27	14	109	49	0	33	24	266
Filinia brachiata (Rousselet, 1901)	0	0	0	0	0	0	0	37	37
RSp1	33	223	467	112	51	0	57	27	970

ANNEX XXXVII - Copepod count along time in M2 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	7/8	Sum
Nauplius Stage	24	151	327	62	91	128	0	0	783
Copepodite Stage	0	0	0	0	0	0	0	0	0
Adult	0	0	0	0	0	0	0	0	0

ANNEX XXXVIII - Ostracod count along time in M2 sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	7/8	Sum
Ostracod	20	0	0	0	0	0	25	0	45

ANNEX XXXIX - Rotifer count along time in R sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Brachionus rotundiformis	0	1076	152	95	330	5904	59	6	103	7725
Brachionus calyciflorus Pallas	4	250	30	25	0	0	0	0	4	313
Brachionus urceolaris O.F. Müller	0	35	14	0	0	0	0	0	0	49
Brachionus angularis	0	102	73	200	3	0	0	0	3	381
Brachionus quadridentatus	7	0	0	4	21	0	4	0	0	36
Keratella tropica sp	0	11	25	0	0	4	0	0	3	43
Keratella cochlearis (Gosse, 1851)	0	0	5	0	0	0	0	9	0	14
Hexartha sp.	0	0	0	0	0	0	0	3	0	3
Ascomorpha sp.	24	0	12	0	6	83	25	5	49	204
Filinia terminalis (Plate, 1886)	0	20	36	18	0	0	0	0	0	74
Filinia cornuta	0	0	0	0	0	0	0	0	17	17
Filinia brachiata	0	0	0	0	0	0	0	0	3	3
M. ventralis sp.	0	0	0	0	0	0	3	0	0	3
Trichocerca sp.	0	0	0	0	17	0	0	0	0	17
Cephalodella forficula (Ehrenberg, 1832)	0	0	0	0	0	0	0	6	0	6
Rsp1	0	36	8	24	33	28	16	13	0	158
Rsp2	0	0	0	11	2	0	0	0	0	13
Rsp4	0	0	0	0	0	20	0	0	0	20
Rsp5	0	14	0	0	0	0	0	0	0	14
Rsp6	0	0	0	0	0	0	4	0	0	4
Rsp9	0	33	14	0	0	0	0	0	0	47
Rsp10	0	40	26	0	0	0	0	0	0	66

ANNEX XXXX - Copepod count along time in R sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Nauplius	15	19	103	133	21	94	2	5	3	395
Copepodite	0	6	10	0	0	0	0	0	0	16
Adult	0	0	0	0	2	0	0	0	0	2

ANNEX XXXXI - Ostracod count along time in R sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Ostracod	0	0	0	0	0	0	7	2	2	11

ANNEX XXXXII - Cladocera count along time in R sampling site.

	19/5	30/5	6/6	16/6	26/6	7/7	17/7	28/7	7/8	Sum
Family Moinidae	0	17	35	5	10	0	0	0	0	67